A SUDANESE ACTINOMYCOSIS

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INTRODUCTION

The whole subject of the organisms causing Actinomycosis is in such a confused condition at the present time that any attempt, no matter how small, which endeavours to simplify their recognition must be of value to workers at disease in the Tropics, and therefore we bring forward the following remarks.

With Pinoy we look upon an *Actinomycosis* as forming a part of the pathological group entitled '*Mycetoma*,' of which we accept the following as a suitable definition:

> 'The term *Mycetoma* includes all growths and granulations producing enlargement, deformity, and destruction, in any part of the body of man, brought about by the invasion of the

affected area by certain species of fungi, belonging to different genera, which give rise to variously coloured and shaped bodies called grains, which are found either embedded in the pathological tissue forming the growths and granulations, or escaping freely in the discharge from the diseased area.'

By the term *grain* or *granum* we do not mean some vague body which may be confused with the mycological expression '*Sclerotium*'; on the contrary, we use these words in the sense defined by Chalmers and Archibald, which is as follows:—

'The word *granum* or *grain* is applied to certain coloured bodies of varying consistence, size, and shape, which are composed of hyphae, and sometimes chlamydospores, embedded in a matrix, and which on germination give rise to mycelial filaments and are found in Mycetomas.'

By the above definitions it is possible to differentiate between the *Mycetomas* and those *Pseudomycetomatous* conditions which are not infrequently seen in the Tropics, and are due to Framboesia tropica, Sporotrichosis, and Angiokeratoma.

The above definition of a Mycetoma covers very wide pathological and aetiological fields, and for purposes of simplification it is suitable to use Pinoy's classification, and to divide the Mycetomas into two classes, as follows:—

A. *The Maduromycoses* are those forms of *Mycetoma* with grains composed of large segmented mycelial filaments, possessing well-defined walls, and usually chlamydospores.

B. *The Actinomycoses* are those forms of *Mycetoma* with grains composed of very fine non-segmented mycelial filaments, in which usually the walls are not clearly defined from the contents, and without chlamydospores.

The term *Pseudoactinomycosis* is used very loosely, but should mean an infection of man due to a *Nocardia* or *Cohnistreptothrix*, which does not come under the definition of an actinomycosis as given above.

In the present communication we are only concerned with the Actinomycoses, of which we propose to discuss a variety found in the Sudan, but before so doing we desire to consider, in the briefest possible manner, the Actinomycoses previously described in man.

HISTORICAL

For purposes of description the history of the Actinomycoses of man may conveniently be divided into four periods, which are:

A. Early History.
B. Madura Foot Period.
C. Mycetoma Period.
D. Actinomycosis Period.

A. *Early History.* According to Waring as quoted by Collas, the Sanscrit work 'Vaweda,' by which is probably meant At'harvavéda, describes a disease of the foot termed *Padavalmicum* which causes swelling and the formation of little fleshy tumours which, after an interval of a year from the commencement of the disease, discharge a peculiar fluid.

This disease is distinguished from another malady of the foot which is called *Slipatham*, or Elephant Foot.

If the above is a correct quotation from the At'harvavéda, then the Ancient Indian Surgeons must have distinguished Elephantiasis of the foot from such conditions as might have been produced therein by Mycetoma, Yaws, etc.

It is, however, curious that, like Collas, we have been unable to find any account of such a disease in the writing of Sušruta.

The term *Perical* used by Kaempfer in 1712 is applicable to any enlargement of the foot, whether caused by Elephantiasis, Mycetoma, or Yaws, but the Pondichèry Missionary of 1714 appears to have seen the disease *Mycetoma*, and possibly the *Actinomycotic* variety, because he describes under the term 'Fourmilière des Vers' an incurable disease of the foot in which numerous small ulcers form which intercommunicate by means of canals full of worms. These canals are described as being peculiar in that if one closes another opens.

Heyne probably recognised some sort of a Mycetoma, in 1806, in the foot of the Rajah's brother at Cuddapah, and Brett's 'Adipose Sarcoma,' described in 1840, may have been of the same nature, but we have been unable to refer to these descriptions with which the early history of the diseases closes.

B. *Madura Foot Period.* With the closing years of the last period it will be noticed that it began to dawn upon the medical

men of India that there existed in that country a peculiar disease of the foot, and this was emphasized by Gill of Madura, who, in 1842, described a condition of that member which was characterised by marked deformity and fungoid excrescences, from which flowed an offensive ichorous discharge, while internally the disease produced a condition resembling fibro-cartilage, and destroyed joints, cartilages and ligaments.

Four years later, Colebrook, Gill's successor at the Madura Dispensary, confirmed these observations, and stated that the disease was commonly known in some parts of India as 'Madura Foot.' As no mention is made, as far as we know, by these authors of any black pigment being present in their cases, we conclude that probably they saw the actinomycotic variety of Mycetoma.

It is curious to note that about this time (1845) von Langenbeck, in Kiel, made illustrations of some curious bodies which he considered to be fungal in nature, and which he found in the pus from a case of Spinal Caries. Unfortunately he never published this observation, which was made known by Israel one year after Bollinger's discovery, which will be mentioned below.

In 1848, Lebert found some peculiar spherical yellowish bodies, about the size of a pin's head, in some thick gelatinous pus which Louis had obtained from an abscess associated with much swelling of the thoracic wall in a man aged 50 years, in Paris. These bodies were carefully examined, both microscopically and chemically, and drawings were made which were subsequently published by Lebert (1857).

We have examined copies of these drawings, and they represent in a typical manner the fungus of an Actinomycosis. Lebert, however, failed to recognise their fungal nature.

In 1855, Smith, in London, made some drawings for Paget of a tumour of the upper jaw, in which an organism resembling a ray-fungus is portrayed. These drawings were published by Kanthack (1896).

Also in 1855, Ballingall, in India, described a disease of the foot in the discharge from which he found bodies composed of large cells with transparent fringes containing irregular spicules, or simply of radiating spicules without cells. In 1858, Rustomji described a variety of Madura foot in which he found small, soft, yellowish granules, and which he distinguished from another variety

of the same disease in which he found a dark, soft, thick substance.

The description of Black Maduromycosis by Godfrey in 1845, and of Actinomycosis by Ballingall in 1855, combined with the differentiation of these two forms of *Madura Foot* by Rustomji in 1858, closes the historical era which we have named the Madura Foot Period.

C. *Mycetoma Period.* In 1860, Vandyke Carter began that series of epoch-making publications which were eventually to lay the foundations of the fungal nature of the black and yellow varieties of Madura Foot. He first became acquainted with the black, subsequently with the yellow, and finally with the pink or red varieties of the disease.

He recognised the fungal nature of the black grains, and inventing the term Mycetoma from the Greek words μύκης a fungus and ὀίδημα a tumour, he named the two principal varieties *Melanoid* and *Ochroid* respectively, and he believed them to be different stages of one disease caused by a mould *Chionyphe carteri* Berkeley 1862, belonging to the Mucorini, which he obtained by placing black grains on cotton soil or rice, although he honestly stated that the fungus had no clear connection with the black grains, but seemed to spring up independently of them. Eventually in 1886, influenced no doubt by the researches which will be detailed below, he gave up *Chionyphe carteri*, and drew attention to the similarity existing between the by-then-known fungus of Actinomycosis and that found in Mycetoma. It may also be noted in passing that Coquerel (1866) in Réunion described and illustrated a case of the Ochroid variety in a man coming from Pondichèry; that Rivolta found rods like those in the retina in 'Mal del Rospo' in cattle in Italy; that Moxon and Hogg, in 1870, noticed fine fibrils in the Ochroid grains of a Mycetoma; that Robin, in 1871, saw crystalline concretions in some pus which he compared with Lebert's illustrations; that Heller, in 1872, observed and described some ray-like bodies in yellow grains from an acute infection in man, and that Perroncito, in 1875, described granular elements considered to be cryptogamic in nature in yellow grains of the Osteosarcoma of the jaw in cattle. All these observations form a natural series leading up to the discovery of the true nature of the causal organism, and thus bring us to the last period of our history.

D. *The Actinomycosis Period.* This period opens with

Bollinger's epoch-making work in 1876 on the lumpy-jaw of cattle, a disease which had been recognised since 1785, and in which he found the constant presence of a branching organism. This fungus was examined by Harz (1877-78), who gave it the name *Actinomyces bovis*, but, most unfortunately, this generic name cannot stand, because, unbeknown to Harz, it had already been used by Meyen (1827) for a fungus which he called *Actinomyces horkelii*, and which is in no way related to the group of fungi which we are considering. This mistake launched the generic name applicable to these organisms on to a sea of change, and led to much confusion.

Genus Nocardia. Bollinger's ray-fungus belongs to a genus of which the correct name is *Nocardia* de Toni and Trevisan 1889, a term derived from Nocard, the celebrated French Parasitologist, who was the first investigator to clearly recognise this fungus in France. We state that it is the correct name for the following reasons:—

1. It is the oldest name against which no objections can be raised.
2. It has been formally adopted by the Botanical Section of the First International Congress of Pathology.
3. The objections to the other names in use are as follows:—
   (*a*) *Streptothrix*, as proposed by Rossi-Doria, cannot be used, as it was originally suggested by Corda in 1839 for *S. fusca*, which is quite a different fungus.

   It was also used in 1875 by Cohn for another organism closely allied to a 'Nocardia,' as we shall see later, so that Cohn's and Rossi-Doria's names can only be utilised as synonyms of the organisms to which they were wrongly applied because of the priority of Corda's name.

   (*b*) *Discomyces* was used by Rivolta in 1878 merely as a trivial name, and though it has not been applied to any other genus, still the word *Discomycetacae* was introduced in 1836 by Fries for a large fungal family and has come into general use, and therefore has the double claim of priority and general use, and as its type genus should bear the name *Discomyces*, confusion is

bound to arise if the same term is retained as the generic name of Bollinger's organism.

(*c*) *Bacterium* was suggested by Affanasieff in 1888, but Ehrenberg had used this name in 1830 for the organisms popularly known as bacteria, and therefore Affanasieff's suggestion falls to the ground.

(*d*) *Oospora*, as utilised by Sauvageau and Radais in 1892, is not available because it is younger than the name 'Nocardia,' and because it was previously used in 1833 by Wallroth for certain fungi previously classified as *Torula* Persoon 1801.

(*e*) *Cladothrix*, as brought forward by Macé in 1897, cannot be used because the name 'Nocardia' has priority, and because it was originally used by Cohn in 1875 for the organism *Cladothrix dichotoma*, which is septate and is only falsely branched, and hence is quite different from Bollinger's fungus.

The genus *Nocardia*, which will be defined later in this paper, contains a large number of species which live saprophytically in soils from whence their spores can be spread by the agency of air or water to sewage, sputum, etc., etc. Some of them have acquired parasitic habits, living in plants in which they cause root tubercles or, in other instances, tumours with ray fungi, thus somewhat resembling the Actinomycosis of animals. They have also been found living in Molluscs and in the alimentary canals of larval insects, as well as in the form of pathogenic fungi in Reptilia, Aves and Mammalia, in which they mostly occur in the Herbivora or in Omnivorous Man, though they are known in the grass-eating dog, but are rare in other Carnivora. Their geographical distribution appears to be world wide.

With reference to their method of entry into the human body, it appears to be often associated with some slight traumatism with some vegetal substance, such as a thorn, while the best treatment is undoubtedly complete removal wherever possible, still partial extirpation associated with treatment by Iodide of Potash, as first advocated for this purpose in 1885 by Tomassen, but in large doses such as ninety grains per diem, as used by Carroll with success in 1905, is also capable of effecting a cure.

For purposes of comparison with the fungus which we are about to describe as the causal agent of a new form of Actinomycosis, it is necessary to briefly review the organisms at present known to cause this disease in man, and we will commence with Bollinger's cattle parasite.

1. *N. bovis.* The correct name for Bollinger's organism is *Nocardia bovis* (Harz 1877) and it appears to have been first seen in man by Israel in 1878. Corre (1883) was the first to draw attention to the similarity between Actinomycosis and the Ochroid variety of Mycetoma, while Acland (1886) was the second observer to demonstrate the presence of Actinomycosis in man, and as Israel's name is associated with quite a different human Actinomycoses, we propose to name this variety *Acland's Actinomycosis.* In 1886, Vandyke Carter, as we have already stated, also drew attention to the likeness between Actinomycosis and Mycetoma. Finally, in 1891, Bostroem grew *N. bovis* from eleven cases of Actinomycosis in man, and since that time it has often been cultivated and described. It is a *Nocardia* with radially arranged filaments which show club-like enlargements of their extremities, caused by a protective thickening of the walls in animals and less commonly in man, and having abundant Gram-positive but not acid-fast hyphae, some of which end in chains of arthrospores.

It grows well aërobically at 22° C., but better at 37° C. Anaërobic growths are, as a rule, but poorly developed.

It may form a dry pellicle on the surface of broth, but more usually it gives rise to cohering colonies at the bottom of the tube, and in either case the medium remains clear.

It grows slowly on gelatine, producing a yellowish-white growth and slow liquefaction, beginning about the seventh day. The resulting fluid may or may not be dark coloured. On blood serum it produces poor growths, and no liquefaction or pigmentation of the medium.

On agar and glycerine agar it forms hard spherical white colonies, which give rise to an undulating crateriform growth, having a yellowish or greyish tint, which in its turn becomes a lichenoid ashen grey or yellowish mass with a powdery efflorescence. On maltose agar it forms discrete fawn-coloured colonies, later becoming yellow, dark brown or even black, while the medium may be slightly darkened.

On potato it forms confluent, hard, raised, variously-coloured masses, at first white but becoming greenish yellow, brown, greyish black or even black, with more or less erosion and pigmentation of the medium to which the growth is very adherent. No diastatic action has been observed.

Litmus milk is first reddened, but later it becomes a clear brown alkaline liquid. It is pathogenic for man, ox, horse, pig and other animals, while experimentally rabbits and guinea-pigs have been infected by intra-peritoneal inoculation.

2. *N. asteroides.* *N. bovis* (Harz 1877) is not the only organism known to cause Actinomycosis in man, for in 1890 Eppinger obtained an organism which he called *Cladothrix asteroides*, and which is now known as *Nocardia asteroides* (Eppinger 1890), from the lesions in a case of pseudo-tuberculosis of the lungs and pleura, with old caseous nodules in the apices and calcareous degeneration of the bronchial and supra-clavicular glands, together with a cerebral abscess which had ruptured into the ventricles. The fungus was Gram-positive and acid, but not alcohol fast, and grew aërobically on laboratory media, and was pathogenic for laboratory animals. It was afterwards recognised by Almquist, in 1890; by Sabrazès and Rivière, in 1894; by Aoyama and Miyamoto, in 1900, in Tokio; by MacCallum, in 1902, in America; and by Schabad, in 1903, in Russia. It is also the same as the fungus described by Musgrave and Clegg (1907) in a case of Mycetoma in the Philippine Islands under the name *Streptothrix freeri*, because in 1908 these authors state:—

'It is identical with Eppinger's organism—and the name given by us in the first publication should fall as a synonym for *S. eppingeri*, the latter having the priority.'

In order to understand this quotation, it is necessary to remember that *S. eppingeri* is a synonym of *N. asteroides*.

In 1909, Lindenberg, in Brazil, isolated a fungus from a case of Mycetoma of the left leg which began in the popliteal space, and to this organism he gave the name *Discomyces brasiliensis*.

He was very careful to separate it from *N. bovis* and from *N. madurae* (*N. indica*), but he does not appear to have done so with regard to *N. asteroides*. We therefore offer a comparison between the two organisms in the following table:—

Nature of test	*N. asteroides* from Musgrave and Clegg	*N. brasiliensis* Lindenberg	Result of comparison
Seat of disease	Mycetoma of foot	Mycetoma of leg	Difference unimportant
Grains	Consistency : dough-like Colour : yellowish white Size : 0·25-0·5 mm. in diameter	Consistency : soft Colour : yellowish white Size : 0·1-0·5 mm. in diameter	No important difference
Clubs	Usually absent	Absent	Agree
Bacillary and coccal forms	Numerous bacillary and coccus-like varieties	Bacillary and coccal forms present	Agree
Optimum temperature ...	Slower growth at 30° C. than at 37° C.	Better growth at room temperature than at 37° C.	Slight disagreement
Anaërobic cultivation	Does not grow	Does not grow	Agree
Broth	Floating flat particles which later fall to the bottom Medium not affected	Small particles which later fall to the bottom of the tube Medium not affected	Agree
Gelatine	No liquefaction	No liquefaction	Agree
Sabouraud's glucose agar at 37° C.	Centre yellow, periphery pink to pinkish white	Colonies rose violet	Slight disagreement
Potato	At first delicate pink, and later yellow ochre centre with pinkish or white periphery ; the medium becomes darkened	At first a rose colour, and later a yellow orange colour ; the medium becomes brown	Agree
Serum	Growth slower Colonies at first white, later diffuse pink	Grows very badly at 37° C. Colonies white	Later pink not mentioned in *N. brasiliensis*
Milk.....	Yellowish mass No coagulation	Yellowish orange pellicle No coagulation	Agree

The inoculations into animals are not comparable, as Lindenberg did not use monkeys. He was unsuccessful with a guinea-pig, but does not say how he inoculated it, while Musgrave and Clegg were successful by means of intraperitoneal inoculations.

The differences as set forth above between *N. brasiliensis* and *N. asteroides* appear to us to be very slight, and therefore we are able to agree with Pinoy in his belief that they are one and the same organism.

Also Cranwell, Bachmann, and Del Pont (1909) gave an excellent and well-illustrated description of a yellow Mycetoma in Buenos Aires. Unfortunately, they did not grow it on inspissated blood serum, but, as far as we understand their account, we should classify this organism, which they did not name, as *Nocardia asteroides*.

Nocardia asteroides possesses Gram-positive, acid but not alcohol-fast hyphae, which are without club-like enlargements. It produces restricted growths aërobically and usually anaërobically at 22° C. and 37° C., but nothing is stated in the literature we have consulted with regard to any odour arising from these cultures. It does not liquefy gelatine or blood serum, nor has it any diastatic action. It reddens litmus milk, which later becomes alkaline, but is not coagulated or cleared. It grows on the agars and on potato, producing reddish (often brick red) growths. It is pathogenic for monkeys, rabbits, and guinea-pigs.

3. *N. liquefaciens.* This fungus was obtained by Hesse in 1892 from a man in Germany with a left inguinal abscess which communicated with the rectum. Subsequently other abscesses formed on either side of the dorsal spine. The pus from these abscesses discharged soft yellowish grains about the size of a millet seed, which contained a Gram-positive fungus which did not possess clubs. On cultivation it grew readily, and was found to be strictly aërobic. In gelatine stabs it formed a nail-shaped growth, which at room temperature in Europe was only visible on the third day, while liquefaction, beginning on the fourth or fifth day, was complete by the end of the week. The liquefied gelatine was not discoloured, and if the growth stuck to the glass it was yellowish with a whitish covering. On blood serum it formed small cloudy granules of the same colour as the medium, in twenty-four to forty-eight hours. Liquefaction begins at the end of the first week and proceeds slowly,

the liquid remaining quite clear and colourless, and only after some six months turning to a reddish-yellow colour. In broth it forms delicate flakes which fall to the bottom of the tube, and consist of a lower surface which is yellowish white, and an upper surface which is snow white. The medium remains quite clear. No surface growth is mentioned.

On agar the colonies at first form separate rosettes, which remain distinct for a time. These colonies appear to resemble the gelatine culture, being yellowish below and having a white envelope. The growth on glycerine agar is more vigorous than on ordinary agar.

On potato it forms small yellow nodules by the second day, which later become covered with a snow-white efflorescence, which does not alter. Apparently it was not grown on glucose agar, milk or eggs. Intravenous, intraperitoneal and subcutaneous injections into rabbits, guinea-pigs and white mice were negative.

Hesse gave it the name *Cladothrix liquefaciens*, which now becomes *Nocardia liquefaciens* (Hesse 1892), and it appears to be the same organism as that named *Streptothrix buccalis* by Goadby in 1903, and found by him in 1899 in the mouth in cases of pyorrhoea. Goadby's form showed clubs, or club-like swellings. It precipitated the casein in milk, which became clear.

4. *N. indica* (Kanthack 1893), studying specimens of Black and Yellow Mycetoma which came from India, concluded that the latter variety was a true Actinomycosis, and attempted to show that the former was the same, only in a degenerated condition.

He only examined the specimens microscopically, as no cultures were possible, and named the fungus in question *Ooospora indica* Kanthack 1893, calling his two varieties *O. indica var. flava* and *O. indica var. nigra*. The name of this fungus, translated into more modern nomenclature, becomes *Nocardia indica* (Kanthack 1893).

Boyce and Surveyor (1894) clearly proved that the Melanoid variety was due to quite a different fungus from that causing the Ochroid variety, which latter they considered in some, but possibly not in all, cases, to be an Actinomyces, a conclusion in which they were supported by Hewlett and by Boccaro. The latter analysed one hunderd cases of Madura Foot, of which the vast majority were Black Mycetomas, while seventeen had evidence of pricks with an

acacia (Babul) thorn, in several of which it was found present on examination.

Kanthack's name appears to have been overlooked, but it certainly has priority as regards the fungus of an actinomycotic nature causing the Ochroid variety of Mycetoma as seen in India, but difficult of recognition in that it was not cultivated.

In 1892, Gémy and Vincent described a parasitic disease of the foot in Algeria, which they considered analogous to, if not identical with, the Ochroid variety of Vandyke Carter's Mycetoma.

In 1894, Vincent, still working in Algeria, met with a Streptothrix in a similar case. This fungus, which was first known as *Streptothrix madurae* Vincent 1894, is believed to be identical with the fungus found by Boyce in London in an agar tube inoculated in India from a case of the Ochroid variety of Mycetoma.

This Streptothrix found by Boyce is of course an entirely different organism from the mucor-like fungus called *Chionyphe carteri* mentioned above, which therefore cannot be placed in the list of synonyms of *N. madurae*, as has been done by some authors.

Boyce's culture showed a fungus without club-shaped extremities which grew very slowly on agar, glucose-agar and glycerine-agar, at a temperature varying between 35° C. and 37° C. No formation of pigment was observed, but it was remarked that the organism closely resembled that of Actinomycosis.

In 1904, Cornwall reported the cultivation of Vincent's organism in India. He washed the grains in six changes of sterile salt solution, and then planted them on agar in tubes. After an interval of one or two months a growth appeared, which in some cases assumed a pink colour and in others remained a dull white. In subcultures it grew more freely, preserving its characteristics, one of which was to adhere so closely to the medium that each nodule had to be literally dug out when it was required to transfer it to another tube. Puff balls formed in broth and hay infusions, while it was noted that the fungus required plenty of oxygen for its growth and only occasionally formed the pink pigment.

This description by Cornwall leaves no doubt in our minds that he met with Vincent's organism in a case of the Ochroid variety of Mycetoma, and if this is correct, then Kanthack's name assumes its priority and Vincent's becomes a synonym, and the correct name of

the fungus is *Nocardia indica* (Kanthack 1893), and this is supported by Strong's culture of the same fungus from an Indian Mycetoma in 1908.

With regard to the remaining history of the fungus, it should be noted that in 1898 Legrain, and in 1899 Brault, again described its presence in Algeria, while in 1901 Albertini and Desvernine reported its presence in Cuba, and in 1902 Brumpt discovered that it existed in Abyssinia, while Sommer y Greco demonstrated its presence in the Argentine in 1904, and Williamson in Cyprus in 1905, in which year Brumpt, in his classic on Mycetomas, stated that he had obtained it from India, Somaliland and Senegal.

In the same year, Pelletier described a case of Mycetoma with red grains which he saw in Saint Louis, in Senegal. The grains were very small, from 0·4 to 0·5 of a millimetre in diameter, and of a beautiful vermilion red colour. In the same year, Laveran published a paper upon Pelletier's Mycetoma, in which he says that it was possible on making sections of the tumour to easily discern therein little red spots of variable size which stood out from the surrounding neoplasm. These grains contained a large number of Gram-positive micrococcal-like bodies embedded in a ground substance. These bodies, which measured 0·7 microns in diameter, were never found isolated, but always in masses or short chains. No trace of a mycelium could be seen, and for this reason he gave it the name of *Micrococcus pelletieri* Laveran 1906. But coccal-like forms are commonly found in Nocardial infections, and in 1912 Thiroux and Pelletier reported that this red Mycetoma was fairly common in Senegal, where one of them had met with eight cases, from one of which, a suppurating tumour of the right side of the chest, they obtained cultures on Sabouraud's gelatine which very much resembled those of *N. madurae*, but differed therefrom in the following particulars:—

1. The growths were ruby red from their commencement.
2. It had only so far been grown on Sabouraud's gelatine.
3. The growths did not penetrate into the gelatine, and were easily detached.
4. In the parasitic stage the organism takes the form of a Micrococcus in Zooglea.

They renamed the parasite *Oospora pelletieri*. In the discussion

on this paper, Laveran agreed with Thiroux and Pelletier's finding, and Pinoy pointed out that the only real differences between it and *N. madurae* were the greater intensity of the red colour and the more abundant sporulation. Further, he suggested that the correct name was *Nocardia pelletieri*. Under these circumstances, *N. pelletieri* becomes simply a synonym of *N. indica*, of which the full list of synonyms will be given later.

Clegg and Hobdy (1916) described *N. indica* in a native woman in Hawaii.

Nocardia indica, with yellow or red grains, possesses Gram-positive but not acid-fast hyphae, without clubs. It forms restricted growths under aërobic surroundings at 22° C. and 37° C., but will not grow under strict anaërobic conditions. The cultures are without any distinct odour. It is usually said not to liquefy gelatine or blood serum, but Koch and Stutzer say that it has a peptonising effect after a long time. Milk is not coagulated, but after some time is cleared. Pinkish colonies are produced on the agars and on potato. It is non-pathogenic for animals, as far as is known.

5. *N. garteni*. Garten (1895) met with an organism in cases of Actinomycosis in man which he called *Cladothrix liquefaciens No.* 2, in order to distinguish it from Hesse's fungus, which he called *Cladothrix liquefaciens No.* 1, but Brumpt, in 1910, altered Garten's name to *Discomyces garteni*, which now becomes *Nocardia garteni* (Brumpt 1910).

This fungus was grown in 1895 by Garten from the lesions of a case of necrosis of vertebrae and ribs, which was associated with abscesses, sinus formation and empyema. The grains were composed of a tangle of ramified filaments without club formation.

The organism was an aërobe which grew easily on various media, producing on gelatine fine greyish-white points. On the fourth day liquefaction commenced, and was completed by the eighth day. Nothing is said as to the liquid being coloured in any way, and, therefore, we must assume that it was not tinted. On agar, glycerine and glucose agar it formed a greyish-white growth, which became somewhat wrinkled on the surface after two to three days. The wrinkles are deep folds on glycerine agar.

On serum it forms a white layer, which becomes wrinkled and folded after forty-eight hours, when commencing liquefaction may

be noted. On the third day the liquid has increased considerably, and by the sixth day the whole serum is reduced to a perfectly clear fluid. On potato it gives rise to white colonies, while the surrounding medium becomes greenish in colour. It apparently was not grown on eggs, milk, broth, or peptone solutions. It is pathogenic for rabbits and guinea-pigs.

6. *N. krausei.* In 1899, Krause found an organism in an abscess of the lower jaw, in a man in Germany, which was characterised by having long and short rods and club-like forms resembling the diphtheria bacillus.

It did not grow at 22° C. nor on gelatine or potato, but it was a facultative anaërobe which formed slightly yellowish colonies on glycerine agar and was not pathogenic for rabbits, guinea-pigs or mice.

This fungus was named *Streptothrix krausei* by Chester 1901, which name has become changed to *Nocardia krausei* (Chester 1901).

Allied to, or identical with, this species are the fungi causing the conditions described by Mosetig-Moorhof, Dor, and Poncet, and often called 'Pseudo-Actinomycosis' or the Mycoses with yellow grains, which are larger than those of the ordinary Actinomycosis, while they are less numerous in the pus. Microscopically they show a tangle of filaments longer and larger than those of ordinary Nocardias, between which lie micrococcal-like débris. They never show clubs at the periphery and do not grow on solid media like gelatine. They grow quickly in broth, forming a skin on the surface. Cultures on serum give clavate forms like the diphtheria bacilli.

The fungus causing the above conditions was named *Nocardia ponceti* by Verdun in 1913, and may be a synonym for *N. krausei* (Chester 1901) for the following reasons:—

A. The Pseudomycetomatous condition of Poncet does not differ from the definition of Actinomycosis given at the commencement of this paper.

B. *N. ponceti* only differs from *N. krausei* in the following details.

1. *Broth is rendered turbid and has a bad odour*, but Foulerton has pointed out that this turbidity, together with the odour which was described as being associated

with these growths, may have been due to the pus not being collected aseptically, and therefore the turbidity and odour may have been due to contamination, as in addition to these characters *N. ponceti* forms a typical puff ball, just like *N. krausei*, in which the turbidity and odour are absent.

2. *According to Verdun, it does not grow on agar.* It is not known whether *N. krausei* grows on plain agar, but it can grow on glycerine agar and (according to some authors) on glucose agar.

C. They resemble each other in:—

1. Morphology.
2. They both possess clavate forms like the diphtheria bacilli.
3. Both grow on serum.
4. Neither grows on gelatine.

Other reactions are given for one, but not for both organisms, and are therefore useless for purposes of comparison.

We therefore at present see no reason why *N. ponceti* should be considered as a species distinct from the older *N. krausei*, of which its name becomes a synonym.

7. *N. somaliensis.* Bouffard observed two cases of a Mycetoma at Djibouti in French Somaliland, which appears to be peculiar both in its histological appearances and in its cultural characters. Brumpt, in 1906, classified this fungus in his new genus, *Indiella* Brumpt 1906, calling it *I. somaliensis*, and pointing out that, judging by the descriptions given by older writers in India of the macroscopical appearances of some of the Ochroid varieties of Mycetoma, this variety might be found to be more common than Vincent's *N. maduræ* (= *N. indica* of Kanthack).

Balfour (1911) reported the presence of the same causal agent in a case of Mycetoma of the hand in the Anglo-Egyptian Sudan, and gave a photo-micrographic illustration of the growth, and in the same year Fulleborn described and gave excellent illustrations of a case from German South-West Africa, which occurred in a Herero aged twenty years. A study of Fulleborn's preparation induced Brumpt to alter his generic diagnosis for the fungus which in 1913 he classified as *Discomyces somaliensis*, which, converted into our

present nomenclature, becomes *Nocardia somaliensis* (Brumpt 1906), but he is inclined to think that it ought to form a separate genus or sub-genus, for which he proposes the name *Indiellopsis* Brumpt 1913, because it secretes around itself in the grain a hard sheath, insoluble in potash and in Eau de Javelle, which no other Nocardia is known to do.

This year, we met with this fungus in a Mycetoma of the foot, in Khartoum.

The grains are hard, one millimetre in diameter, and being of a reddish yellow colour resemble the eggs of fish. The fungus will not grow on hay, or on dura broth, but it quickly produces a white lichen-like folded growth, becoming yellow on the fifth to sixth day on potato, but this growth never becomes red like that of *N. indica*.

Genus Cohnistreptothrix. In 1891, Wolff and Israel published a beautifully illustrated account of a Streptothrix, which they had isolated from two cases of Actinomycosis in man, viz., from the lungs and from a retromaxillary growth. This organism was considered to differ from *N. bovis* in that it grew best anaërobically, that branching was absent, and that its injections into animals were regularly positive in their result. These three characteristics induced Kruse, in 1896, to make a new species for it under the name *Streptothrix israeli.* In 1911, for reasons presently to be set forth, Pinoy founded a new genus *Cohnistreptothrix*, with Israel's organism as the type species, and therefore its name becomes *Cohnistreptothrix israeli* (Kruse 1896).

It appears to us to be of importance that the reader should clearly understand the nature of the organisms included in this genus, and, therefore, we digress from our main subject in order to give a brief history.

Lachrymal concretions have been known since Césoin described them in 1670. In 1848, Gruby, examining one of these objects, found it to be composed of a fungus, which he believed to be the same as that causing favus, but Cohn, in 1875, examining another such concretion, also saw a fungus, for which he created a new genus streptothrix, calling the fungus in question *Streptothrix foersteri* Cohn 1875, which may be the same organism as *S. aureus* Du Bois de Saint Sévérin 1895, and must be closely related to *Nocardia tenuis* Castellani 1911, which belongs to the same genus, and as its colonies on agar are ' cerebriform ' it may possibly be the same or related to *Streptothrix radiatus* and *S. cerebriformis*, both described from cases of keratitis by Namyslowski in 1909, as well as the more aërobic hyphal form of Silberschmidt's organism.

Unfortunately, a mistake was made, for Cohn was not aware that the name Streptothrix had already been given by Corda, in 1839, for another and quite different fungus, which is known as *Streptothrix fusca* Corda 1839, and which

is to be found in all works of any importance on systemic mycology. Therefore, as Streptothrix is not available, after many changes, the generic name has become *Cohnistreptothrix* Pinoy 1911, and to this genus Israel's human organism belongs. It differs from Bollinger's type of fungus in growing best anaërobically, in being difficult to cultivate, and in not producing arthrospores. Other allied organisms are *Cohnistreptothrix thibiergei* (Ravaut and Pinoy 1909), also found in Actinomycosis in man; *Streptothrix spitzi* Lignières 1903, found in cattle, is probably identical with *C. israeli*, as may be Doyen's Streptothrix; while *Nocardia carougeaui* Gougerot 1909, in juxta-articular nodules, and *Streptothrix cuniculi* Schmorl 1891, probably also belong to this genus, as well as the Streptothrix recently discovered in a liver abscess in America by Bloomfield and Bayne-Jones (1915), as we have consulted the authors upon this point, with which they are in agreement. Perhaps the bacillus described by Sawtschenko, in 1896, as the causal agent of a Pseudomycetomatous condition may also belong to this genus, and it is also possible that the *Coccobacillus pseudo-actinomycosis polymorphus* Berestneff 1898 may be the same as the chromogenic anaërobic streptothrix, obtained from human pus by Neschezadimenko in 1908, and carefully described.

8. *C. israeli.* This organism appears to be of increasing importance in human pathology, for, according to Pinoy, it appears to affect man more often than *N. bovis*. It was first discovered in man, as mentioned above, by Wolff and Israel in Germany, and has since been found in thirteen cases in the United States by Wright. It has also been found in cattle by Lignières and Spitz (1904) in the Argentine, and by Pinoy (1913) in France.

It is composed of short and long rods, some of which show club-like swellings, while in old cultures spores which resemble cocci in appearance can be seen. It grows but poorly in the presence of air, but much better anaërobically at 37° C. on agar, on which it forms dew-like drops, which later become yellowish and generally remain discrete. In broth it forms a deposit of small scaly particles. It does not grow on gelatine at the room temperature of Europe, but egg cultures show typical branched filaments with club-like ends, which later break up into bacillary and coccal forms, but true arthrospores (i.e. resistant spores) are not produced. It forms granulation tumours when inoculated intraperitoneally into rabbits and guinea-pigs, after an interval of four to seven weeks. In these tumours typical actinomycotic grains can be found, containing branched filaments with clavate ends.

9. *C. thibiergei.* This fungus was discovered in 1909 by Ravaut and Pinoy in a case of Actinomycosis which produced generalised subcutaneous and intramuscular nodules in a man in France.

The nodules opened and discharged blood-tinged pus, in which the fungus was seen sometimes in isolated bacillary form and sometimes as very small white grains, which in the tissues might measure some 80 microns and be composed of a radiating mycelium with or without fine club forms. It grows well aërobically and anaërobically, but the former produces more bacillary and the latter more filamentous forms. The optimum temperature is about 37° to 38° C. It does not appear to be pathogenic for laboratory animals.

Actinobacillus. This curious Gram-negative bacillus-like organism, discovered by Lignières and Spitz (1904) in the Argentine, in cattle, has been shown by Griffith to be wide-spread in the world as a cause of Actinomycosis in cattle, in which the grains are very small and possess clubs. It grows well aërobically and anaërobically on various media, and does not liquefy gelatine or blood serum, but it gives rise to indol, and is pathogenic for many animals. It may also, in the future, be found to cause Actinomycosis in man, but we are only acquainted with one human infection, viz., that described in Paris, in 1911, by Ravaut and Pinoy, which occurred in a man from Argentine, causing otitis, mastoiditis and meningitis. It was obtained from the cerebro-spinal fluid, and on glucose peptone formed clubs, and this being so its name would become *Nocardia lignieresi* (Brumpt 1910). It may also have been the causal agent in the Pseudo-actinomycosis described by Cozzolino in 1900, but we have been unable to refer to this author's writings, and, therefore, leave this merely as a suggestion, and, at all events, at present it need not be classified with the organisms causing human Actinomycosis in the sense defined above.

After the description and classification of the organism which we have found here, it will be necessary to compare it with the nine fungi just described as causal agents in cases of Actinomycoses, and therefore we now turn to the description of the Sudanese Actinomycosis and its causal fungus.

THE SUDANESE ACTINOMYCOSIS

The specimen about to be described was in the form of a small roundish fibrous tumour situate on the dorsum of the foot, the skin of which was not affected, in an adult Sudanese man who came to the Khartoum Civil Hospital for treatment. There was no enlargement of the lymphatic glands, no sinuses and no discharge.

The growth was found to be lying in the subcutaneous tissue and was removed entire, and the patient made an excellent recovery, and, so far as is known up to the time of writing this paper and one year after the operation, there has been no recurrence.

The growth was cut into halves, from one of which the yellowish grains were removed for purposes of cultivation, while from the other half (fig. 1) sections were made.

PATHOLOGICAL HISTOLOGY

When fig. 1 is examined, it will be observed that it represents a section of the growth magnified one and a half times, and shows that for purposes of description it can be divided into two portions, viz. :—

1. A dense matrix.
2. A number of irregularly shaped darker bodies, 'the fungal masses,' embedded in the matrix.

The Matrix. When the matrix (fig. 13) is studied by the aid of higher magnifications, it will be seen to be composed of white fibrous connective tissue containing a large number of connective tissue corpuscles, and here and there a blood vessel or a small group of blood vessels which may or may not be associated with a collection of cells (fig. 13), and, in addition, lymph spaces and small collections of fat cells mostly associated with the blood vessels (fig. 13). When these vessels are studied more carefully, some will be observed to be more or less normal, while others show signs of periarteritis (fig. 13) or endarteritis (fig. 14) of varying degree, which produce diminution and even occlusion of the lumen.

Connected with many of these vessels, and often more or less surrounding them, lie dense masses of cells (fig. 13), which when carefully studied (fig. 15) appear to be all mononuclear. They are not all of the same category, however, for some, judging by their nuclei, appear to be derived from the endothelial cells of the vessels. Another type of cells is characterised by a darker staining nucleus, and appearing when cut in certain directions as though it possessed very little cytoplasm, but, when seen more correctly, has a relatively fair quantity of cytoplasm in proportion to the size of the nucleus. The nucleus being placed excentrically, and the cytoplasm being non-granular and not eosinophile, this cell agrees with Unna's description of a healthy *Plasma Cell* as seen in Actinomycosis.

A third type of cell shows a large vesicular nucleus situate excentrically in a relatively large quantity of cytoplasm, which is either eosinophile or contains eosinophile granules, and corresponds exactly with Unna's description of degenerating plasma cells as seen in Actinomycosis.

Fungal Masses. The darker irregular bodies seen embedded in the matrix in fig. 1, if examined by the aid of higher magnification,

can be seen to consist of fibrous tissue and cells surrounding a portion of the fungus (fig. 12), and have therefore, for purposes of distinction, been termed '*Fungal Masses.*'

When a typical fungal mass is examined (fig. 12) by means of a moderately high magnification, it can be seen to be composed of several distinct areas which, working from the fibrous tissue matrix towards the fungus, lie in the following order:—

1. *The Fibrous Sheath.* This is continuous with the fibrous tissue forming the matrix of the whole growth, as already described.
2. *The Fibro-cellular Layers.* Directly under the dense fibrous tissue there lies a thicker or thinner area composed of loose fibrous tissue, containing in its meshes cells and thin-walled vessels; this area may be termed the fibro-cellular layers.
3. *The Cellular Sheath.* Internal to the fibro-cellular layers comes a mass of cells which may be called the cellular sheath.
4. *The Grain.* Situate in the cellular sheath there lies a more or less distinctly or indistinctly striated body, of varying shape, and often with irregular edges, which is the grain, and is composed of the fungus and its surrounding matrix.

It is proposed to postpone the study of the grain until we discuss the aetiology of the growth, but a few words are necessary with regard to the areas.

Fibrous Sheath. When the fibrous connective tissue forming the matrix is examined, in the vicinity of a fungal mass, it will be observed to show collections of cells at intervals.

Fibro-cellular Layers. If figs. 12 and 18 are examined, it will be seen that these layers are composed of loose fibrous tissue, holding in its meshes plasma cells, healthy and degenerating polymorphonuclear cells, giant cells and blood vessels.

With regard to the giant cells, they may be seen to contain fungal masses (fig. 19), or these may be observed (fig. 16) escaping therefrom, or the giant cells may be remarked to be separated from the fungal mass by a little distance (fig. 17) and to be damaged, while polymorphonuclear cells lie near the fungus, and the adjacent layers of the fibro-cellular tissue may be observed to be arranging themselves circularly (fig. 17) so as to circumscribe the new fungal growth, and so to commence the formation of a new fungal mass.

When two fungal masses lie in close approximation to one another without the intervention of dense fibrous tissue, it will be observed that small areas of the fibro-cellular layers adjoining the two masses show signs of granular degeneration.

Another interesting feature, but by no means confined to the fungal masses, is the presence of cells containing one or several, small or large, rounded eosinophile globules (fig. 2). These were called Fuchsin or Russell bodies by Kanthack, and Botryomycotic bodies by Archibald (1911), who published some excellent illustrations thereof in Plates XV and XVI of the Medical Volume of the Fourth Report of these Laboratories. They are depicted in fig. 2, and appear to be a product of the fungus as we have frequently seen them in Nocardial infections lying in cells at a distance from the fungus, in which case they are a great aid in diagnosis as indicating the probable presence of a fungus somewhere. We have also seen them in masses cut longitudinally and lying in lymph spaces. We have observed them, but much more rarely, in Black Maduromycosis. They have been recorded by all workers at Actinomycosis and Maduromycosis since the days of Kanthack, and appear to us to be probably the same material as that forming the club-like dilatation of the extremities of the hyphae in *N. bovis* and other Nocardias, and that they may possibly be a protective substance excreted by the fungus which only under certain conditions consolidates into the eosinophile form demonstrated in fig. 13 and into the clubs of certain species of Nocardia.

The Cellular Sheath. All our observations tend to support Brumpt's view that primarily the fungus is enclosed in a cell which in the younger fungal areas near the older area is always multinuclear (fig. 19).

Further, in the present specimen, there can be no doubt that the fungus is not destroyed by the giant cell, but on the contrary, as shown in fig. 16, grows and escapes therefrom and starts life as a little fungal mass of its own (fig. 17), in which instance the polymorphonuclear leucocytes now appear upon the scene, and the fibro-cellular coat begins to circumscribe the cells and the fungus, while the damaged remains of the giant cell are seen retiring towards the periphery (fig. 17).

Later, the mononuclear cells mentioned above appear, and these

various cells, together with detritus from the destruction of similar cells situate in a granular network, form the cellular sheath of the grain, as shown in our specimens. This description, although varying in detail, does not differ materially from a composite picture such as can be derived by a study of the writings of Carter, Acland, Kanthack, Boyce and Surveyor, Unna, Schlegel, Foulerton, Brumpt, and other authors who have studied the reaction of the body against different species of the genus Nocardia.

An analysis of its findings will be observed to support the view as to the pathogenesis of the structures found in Actinomycosis set forth by Brumpt in 1905-1906.

AETIOLOGY

The removal intact of the small growth completely cured the condition, therefore the aetiological factor must have been contained therein.

The only feature of the growth which cannot be attributed to the reaction of the body is the grain, and as this is composed of a fungus and its products, and as all the steps of the bodily reaction can be traced seriatim to be directed against this fungus, and against it alone, it becomes sufficiently manifest that this organism is the cause of the disease, even though the final proof of the reproduction of the disease by inoculations of cultures into animals is wanting, as all our experiments have been negative.

The points requiring investigation with regard to the aetiology of the Sudan Mycetoma may be classified into :—

1. The histology and mycology of the grain.
2. The cultivation of the fungus.
3. The mycology of the cultures.
4. The classification of the fungus.
5. Animal inoculations.
6. Comparison with the fungi known to cause other forms of Actinomycosis.

1. *The Grain.* As the skin covering the tumour was unbroken, and there was no discharge, grains could only be obtained by removal from the portion of the specimen set aside for this purpose.

A grain so obtained, and magnified twenty times, is depicted in

fig. 3. It was a soft, smooth, lobulated, yellowish body with a slight tinge of an orange hue mixed with the yellow, and measured about 0·5 millimetre in diameter. To be more exact, it resembled Ridgway's Color Standard, Plate III, 17 O-Y, f, Pale Orange-Yellow.

When a portion was flattened out between a slide and cover-glass, and then examined by means of a fairly high magnification, it was seen that the grain was composed of typical nocardial bacillus-like hyphae (fig. 6) embedded in a more or less homogeneous matrix. The width of a given hypha was less than one micron, and no definite thick hyphal wall, such as is easily discerned in a fungus like Aspergillus, could be detected, nor could any septa be seen.

On examining sections stained by haematoxylin and eosin, it is observed that the fungal matrix is stained by the haematoxylin, while the hyphae remain as clear, radiating, unstained branched spaces. The margin of the grain does not possess a sheath (fig. 18), and may be even or may be irregular, having well defined processes jutting into the cellular sheath, but is in any case closely attached to polymorphonuclear cells, or in much younger grains to these and giant cells. The hyphal filaments are well coloured by the Gram-Weigert method, and are then seen to be branching or bacillary-like in appearance and to contain homogeneous or beaded contents. No clubs or club-like ends could be detected, on the contrary the ends were thin and rounded.

It is, therefore, evident that the grain is composed of branching, exceedingly fine hyphae, not markedly septate and without the usual thick and well defined walls of the typical fungal hypha, and containing either homogeneous protoplasm or this with darker staining bodies at intervals, and embedded in a more or less homogeneous matrix.

2. *Cultivation.* The fungus obtained from the grains described in the preceding section grows fairly well at 22° C., best at 30° C., moderately well at 37° C., and not at all at 60° C. under aërobic conditions (fig. 8). It also grows well under anaërobic conditions (fig. 9), but the white efflorescence is more marked than upon aërobic growths. The optimum medium and temperature appear to be Blood Serum at 30° C., i.e. upon an alkaline medium. It appears to prefer alkalinity, as potato has to be rendered slightly alkaline

before it will grow well. It is Gram-positive, but not acid-fast, as tested by acid decolorization. Its cultures have no distinct odour, and are usually warm buff in colour (i.e. Ridgway's Standards, Plate XV, 17 O-Y, d) when well developed and free from efflorescence.

In Peptone Broth at 37° C., it forms numerous white non-cohering flocculi which sink to the bottom of the tube, while the medium remains quite clear and unpigmented, and there is no growth on the surface.

In Glucose Peptone, at 37° C., the growth resembles that in Peptone Broth.

In Litmus Milk, at 30° C. and at 37° C., it grows well, but it neither acidifies nor clots this medium at any time nor does it form a surface growth, but it appears to increase the alkalinity of the medium.

In Nutrient Gelatine, in stab cultures incubated at 22° C., it forms a surface growth in the form of small rounded light buff colonies, while along the depth of the stab for its entire length it gives rise to numerous minute colonies at the end of nine days. These growths gradually become smaller and smaller as the distance from the surface into the depth of the gelatine is increased. It continues to grow in gelatine long after the eighth day after inoculation, but it never causes liquefaction or pigmentation of the medium.

On Agar-Agar, if the medium is alkaline, it forms the typical warm buff-coloured convoluted growth in forty-eight hours at 37° C., and is often surrounded by secondary younger colonies. At 30° C., in the same time, the convoluted growth is more marked, but is much paler in colour, while there are but few secondary isolated young colonies (fig. 28). It is not adherent to, nor does it pigment the medium; it produces no smell. If the agar is acid no growth takes place.

On Clear (not Sabouraud's) Maltose Agar, at 30° C. and 37° C., it gives rise to raised, rounded or oval, corrugated, moist light buff coloured colonies (fig. 5), which when young have a radiating fringe of white rays, but when old possess only a glistening light whitish margin level with the surface of the medium into which the growth does not penetrate and which does not become pigmented.

On Glycerine Agar, at 37° C., it forms a raised, translucent, moist, warm buff coloured growth, which, however, is not as luxurious as upon maltose agar. A given colony upon this medium has clear cut edges or a whitish margin lying nearly level with the surface, and does not sink into nor pigment the medium.

On Glucose Agar (fig. 29) it produces in three days at 30° C. a warm buff coloured growth which is markedly convoluted, somewhat resembling the convolutions of the brain after the removal of the pia mater. The medium is not pigmented.

When grown on *Sabouraud's Maltose Agar* it produced a typical growth, which is depicted in fig. 23, but it was more radiated than convoluted, and had well marked white efflorescence.

On Sabouraud's Preservative Medium it grows well under aërobic (figs. 4 and 8) but not so typically under anaërobic conditions (fig. 9) at 30° C.

On Inspissated Ox Blood Serum it grows well, forming in seven days at 30° C. light buff coloured, raised, coiled colonies (fig. 10), which, after twelve days' growth at the same temperature, begin to liquefy and clear the medium (fig. 11), which they do not pigment.

On Potato it grows extremely well at 30° C. to 34° C., forming a light buff coloured, raised, convoluted, moist growth, on which a white efflorescence begins to form after nine days. At 37° C., in about forty-eight hours, it produces a typical convoluted growth, which in four days becomes surrounded by many small colonies showing a whitish efflorescence (fig. 7). The medium is neither eroded nor pigmented.

On Carrot it grows well, giving rise to a light buff coloured, raised, corrugated, moist growth, which neither erodes nor pigments the medium.

In Starch Peptone Medium it grows well, but shows no diastatic action after nine days' incubation at 37° C., a control tube being used.

In Sugar Media there was a good growth of the puff ball variety, but no formation of acid or gas as tested qualitatively. The sugars used were glucose, lactose, maltose, saccharose, raffinose, salicin and mannitol.

3. *Mycology.* Whether growths or grains are examined, the outstanding feature of the mycology is that the fungus consists of a

branching mycelium composed of exceedingly thin hyphae, generally less than one micron in diameter, without the usual thick hyphal wall which one is accustomed to associate with fungal filaments. These hyphae may contain homogeneous Gram-positive cytoplasm, but more commonly they exhibit a beaded appearance, with areas of intense staining separated by non-coloured (fig. 22) or almost colourless intervals.

The hyphal threads may be of considerable length, as, for example, the one depicted in fig. 20, but they fail to show the septa so commonly met with in fungal hyphae. When old, the cytoplasm of a hypha breaks into fine granules (fig. 25), which, becoming absorbed, leave an empty sheath, which (fig. 24) ceases to retain Gram's stain and becomes tinged with the counter stain. This sheath breaks (fig. 24) and disappears, and now the areas retaining the stain become separated as longer or shorter rods (fig. 21), which resemble bacilli, especially the diphtheria bacillus, in appearance. As these rods can, without doubt, give rise to new mycelial threads, they appear to us to be analogous, though perhaps not homologous with Thallospores.

Hyphal filaments, however, generally show branching which is not dichotomous but irregular in arrangement (fig. 21), and after a certain amount of growth they often give rise to hyphae which produce rows or chains of spores (fig. 26), which form the whitish efflorescence often seen on growths. These spores being Gram-positive, rounded bodies, about a micron in breadth, closely resemble cocci, and, therefore, an old growth with its beaded bacillary forms and its rounded spores resembles most closely a collection of bacilli, micrococci and streptococci, and for such the fungus is sometimes mistaken even at the present.

The Thallospore-like hyphae may be the agency by which the numerous small colonies form round a parent colony, as seen in fig. 7, while the rounded spores appear to us to represent arthrospores, and presumably help to keep the fungus in existence in times of difficulty. They are shown sprouting in fig. 27.

Beside these two kinds of reproduction we have failed to find any reproductive mechanism, and the life cycle from the arthrospore giving rise to the hypha, which breaks into segments and forms a bacilliform mycelium on which the streptococcus-like chain

spores arise, corresponds exactly with the admirable descriptions of the life cycle of a typical parasitic Nocardia, as given and fully illustrated by Foulerton, and need not detain us here, and, therefore, we will pass on to consider the exceedingly difficult problem of the classification of this fungus, which appears to represent in itself the spora! types of Thallospore and Arthrospore.

4. *Classification.* As the fungus in question does not possess known sexual cells, even though its hyphae do not exhibit the usual septa, it must be classified among Schröter's *Eumycetes*, and not among De Bary's *Phycomycetes*.

As its spores are not situate in asci or basidia, it belongs to Fuckel's class of the *Fungi Imperfecti*, and as no accessory fructifications in the form of open or closed receptacles have been observed in any of our cultures, it must belong to the sub-class named *Hyphales* Vuillemin 1910, which is divided into four orders according to the following scheme:—

A. Mycelium composed of fine bacilliform hyphae, usually one micron or less in diameter, with a thickened hyphal wall and septa	Order 1 *Microsiphonales* Vuillemin 1912
B. Mycelium composed of hyphae, usually greater than one micron in diameter, and usually with a thickened hyphal wall and septa:—	Order 2 *Thallosporales*
I. Reproduction by thallospores	Vuillemin 1910
II. Reproduction by hemispores	Order 3 *Hemisporales* Vuillemin 1910
III. Reproduction by conidia	Order 4 *Conidiosporales* Vuillemin 1910

A consideration of this table shows quite clearly that the fungus which we are considering belongs to Vuillemin's order *Microsiphonales*, which contains at present only two genera, which are distinguished as follows:—

A. Grows easily aërobically, and produces arthrospores	Genus 1 *Nocardia* De Toni and Trevisan 1889
B. Grows best anaërobically, but can grow aërobically, usually difficult to cultivate, and does not produce arthrospores	Genus 2 *Cohnistreptothrix* Pinoy 1911

Again there is no difficulty as to the classification, as the fungus which we are studying obviously agrees with the definition given above for the genus *Nocardia* De Toni and Trevisan 1889, but now the difficulty of the specific determination begins, and a very real and serious difficulty it is, and in attempting to make order out of the chaos into which the species of the genus have got, we have availed ourselves of Foulerton's broad lines of classification which, in our opinion, are of great value. It would merely weary the reader if we recorded the difficulties which we have met with in this task, or if we even gave the reasons for the classification which we bring forward. For our present purposes, it will suffice if we give the mere outlines of our results.

With Foulerton (1905-1912) we divide the species of the genus Nocardia into three *sections*, as follows:—

A. Habitat.—Soil, can be found in air or water ...	Section 1 *Saprophytica* Foulerton 1910
B. Habitat.—Plants or animals	Section 2 *Parasitica* Foulerton 1910
C. Habitat.—Soils, plants or animals, but imperfectly described	Section 3 *Incertae sedis*

As our parasite has only so far been found in man, we may, for the time being, confine our attention to the second section to which it obviously belongs, though of course it might be a saprophytic species which had become parasitic in and pathogenic to man, but this question we will leave till later.

The Parasitic Section we have classified into three sub-sections, as follows:—

TABLE I

Diagnosis of the Sub-sections of the Section *Nocardia parasitica*

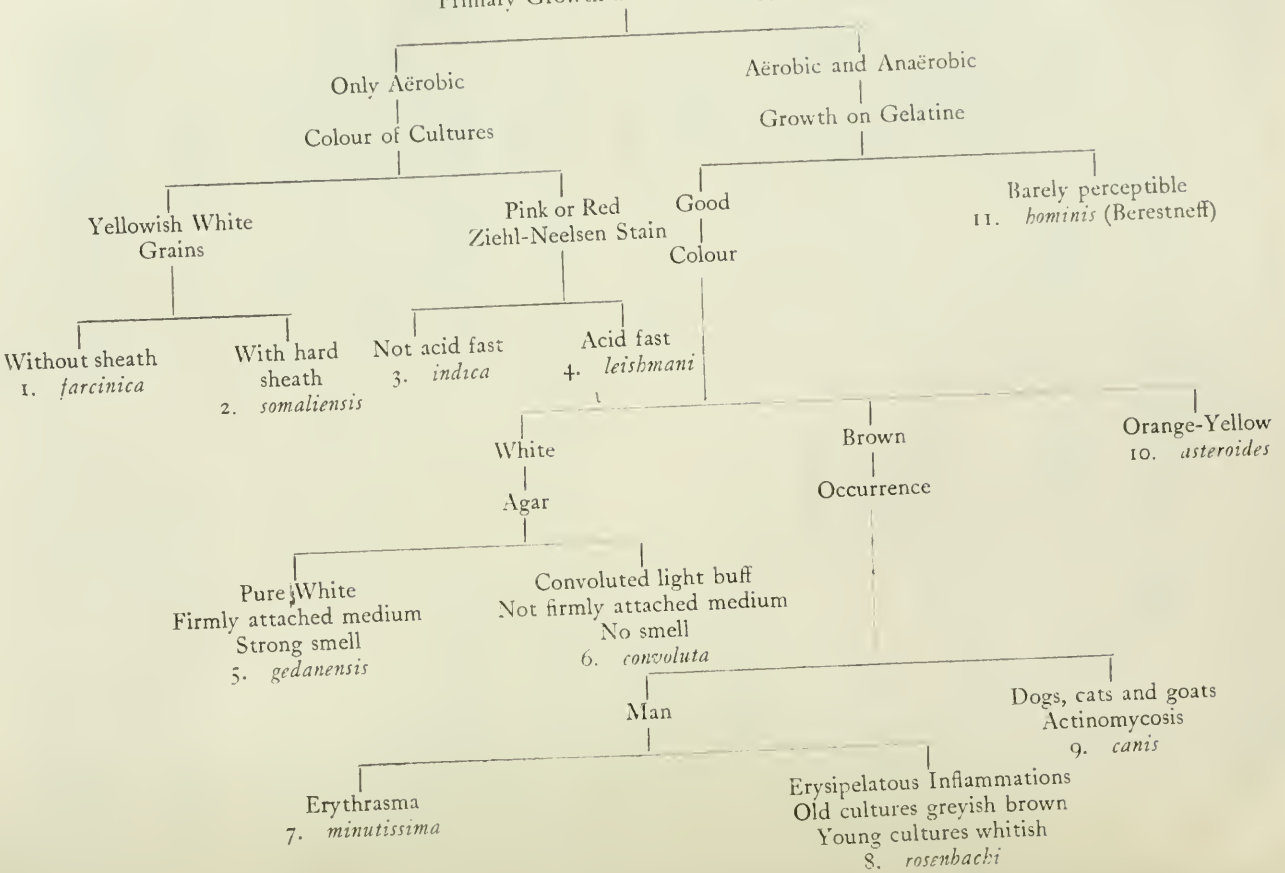
No.	Test	Sub-section 1 Majora	Sub-section 2 Minora	Sub-section 3 Brevis
1	Cultivation at 22° C. and 37° C.	Easy	Not difficult	Difficult at 37° C. Usually nil at 22° C.
2	Growth	Spreading	Circumscribed	Slight
3	Efflorescence	Bright chalky	Dull powdery	Usually absent
4	Hyphal branching	Well marked	Poorly marked	Rare, hyphae often bacilliform
5	Acid fast species	Rare	Common	Rare
6	Odour of cultures	Earthy or mouldy	Absent or faintly as 1	Sometimes faeculent
7	Liquefaction of gelatine and blood serum	Often present	Rare, and usually only one liquefied	Often very slight indications
8	Potato	Growth	Usually growth	Often no growth
9	Diastatic action	Often present	Usually absent	Not known

Our fungus agrees well with Sub-section 2, Minora, of the Section *Nocardia parasitica*.

The species with which we are acquainted, and which we believe belong to this sub-section, can be differentiated according to the following table, in which the Sudanese variety is inserted under the name *Convoluta*, which will be explained below:—

TABLE II

Diagnostic Table of the Species of Section *Parasitica*, Sub-section *Minora*



List I

Synonyms of the species tabulated in Table II:

1. *Nocardia farcinica* Trevisan 1889
   - Bacillus du Farcin Nocard 1888
   - *Streptothrix farcinica* Rossi-Doria 1891
2. *Nocardia somaliensis* (Brumpt 1906)
   - *Indiella somaliensis* Brumpt 1906
   - *Indiellopsis somaliensis* (Brumpt 1913)
   - *Discomyces somaliensis* Brumpt 1913
   - ? Ballingall's Disease
3. *Nocardia indica* (Kanthack 1893)
   - *Oospora indica* Kanthack 1893
   - *Streptothrix madurae* Vincent 1894
   - *Discomyces madurae* Vincent 1895
   - *Nocardia madurae* R. Blanchard 1895
   - *Micrococcus pelletieri* Laveran 1906
   - *Oospora pelletieri* Thiroux and Pelletier 1912
   - *Nocardia pelletieri* Pinoy 1912
   - *Nocardia rivierei* Verdun 1912 ?
4. *Nocardia leishmani* new name
   - New acid fast Streptothrix pathogenic to Man and Animals described by Birt and Leishman in 1902
5. *Nocardia gedanensis* (Scheele and Petruschky 1897)
   - *Streptothrix gedanensis* I Scheele and Petruschky 1897
6. *Nocardia convoluta* new species
7. *Nocardia minutissima* (Burchardt 1859)
   - *Microsporum minutissimum* Burchardt 1859
   - *Trichothecium* J. Neumann 1868
   - *Microsporon gracile* Balzer 1883
   - *Sporotrichum minutissimum* Saccardo 1886
   - *Microsporoides minutissimus* Neveu-Lemaire 1908
   - *Discomyces minutissimus* Brumpt 1910
   - *Oospora minutissima* Ridet 1911
   - *Nocardia minutissima* Verdun 1912
8. *Nocardia rosenbachi* (Kruse 1896)
   - *Streptothrix rosenbachi* Kruse 1896
9. *Nocardia canis* (Rabe 1888)
   - *Cladothrix canis* Rabe 1888
   - *Streptothrix caprae* Silberschmidt 1899
10. *Nocardia asteroides* (Eppinger 1890)
    - *Cladothrix asteroides* Eppinger 1890
    - *Streptothrix eppingeri* Rossi-Doria 1891
    - *Oospora asteroides* Sauvageau and Radais 1892
    - *Nocardia asteroides* R. Blanchard 1895
    - *Streptothrix hominis* Sabrazès and Rivière 1895
    - *Actinomyces asteroides* MacCallum 1902
    - *Discomyces asteroides* Brumpt 1906
    - *Streptothrix freeri* Musgrave and Clegg 1907
    - *Discomyces brasiliensis* Lindenberg 1909
    - The organisms described by Ferré and Faguet, by McCallum, by Schabad probably belong to this species.

11. *Nocardia hominis* (Berestneff 1897)
Nec *Actinomyces hominis* Bostroem synonym of *N. bovis*
Nec *Actinomyces hominis* Affanasieff = *N. bovis*
Nec *Actinomyces hominis* Wolff and Israel = *N. israeli*
Nec *Streptothrix hominis* Sabrazès and Rivière = *N. asteroides*
Nec *Streptothrix hominis* Hayo Bruns 1899
Nec *Streptothrix hominis* Foulerton 1902
Nec *Streptothrix hominis* II Foulerton 1910
Nec *Streptothrix hominis* III Foulerton 1905 = *N. bovis*
Nec *Streptothrix hominis* IV Foulerton 1910 = *N. bovis*
Nec *Streptothrix hominis* III Foulerton 1910

As there is so much confusion with regard to the specific name '*hominis*,' we propose that :—

S. hominis Bruns be changed to *Nocardia bruni*
S. hominis Foulerton be changed to *Nocardia foulertoni*
S. hominis II Foulerton be changed to *Nocardia londinensis*
S. hominis III Foulerton be changed to *Nocardia appendicis*

This table shows that the Sudan fungus (called in the table *Convoluta*) can be differentiated from its allies, but it may be said that we have not separated it sufficiently from the various species belonging to the Sub-sections Brevis and Majora, and, in order to meet this requirement, we give an Appendix of Diagnostic Tables and Lists of Synonyms at the end of this paper, in which Tables III and IV and Lists II and III will enable the reader to understand our views as to the differential diagnosis and synonyms of the various species, belonging to these two sub-sections, with which we are acquainted.

In order to show that our Sudanese fungus is not a known saprophytic species, we have followed Foulerton and divided the Section *Saprophytica* into two Sub-sections, viz., Minora and Majora, with the same characters as for the Section *Parasitica*, excluding only the acid-fast character.

We have further differentiated the species belonging to these two sub-sections in Diagnostic Tables VI and VII of the Appendix just mentioned, and we give the synonyms in Lists IV and V of the same Appendix.

A study of these tables will show that the Sudanese fungus is not similar to any species contained therein.

In order to complete the differentiation, we also provided in List VI the names of a few species of Nocardia belonging to Section 3, *Incertae Sedis*, while in Table VIII and List VII we give

the differentiation and the synonyms of the species of the genus *Cohnistreptothrix*.

These tables and lists do not profess to be final, or even full, but merely to contain such species as we have been able to find in the limited literature at our disposal in Khartoum, and very many more must exist scattered in Medical, Veterinary, Agricultural and Botanical writings.

They are intended to show that, as far as we know, the species of *Nocardia* found in the Sudanese Actinomycosis is new to human parasitology, and also new to science, and, therefore, we name it *Nocardia convoluta* Chalmers and Christopherson 1916, and derive the name from the latin '*convolutus*,' signifying twisted, because of its peculiar growth on potato, blood serum and the agars, as depicted in figs. 7, 10, 11, 28 and 29, and we define it as follows:—

> '*Nocardia*. Gram-positive but not acid-fast and without club formations, found parasitic in man, easy of cultivation and growing aërobically and anërobically at 22° C. and 37° C., with a marked preference for alkaline media, and with the production of good but limited growths on the different agars, and the same at first on blood serum and potato, on which, however, it becomes more profuse later. Not liquefying gelatine, but causing liquefaction of inspissated ox blood serum, and without diastatic action. Colonies usually somewhat translucent when young, and of a light to warm buff colour (Ridgway's Plate XV, 17, O-Y, f or d), and either convoluted or having the appearance of a jelly turned out of a mould, but later developing a whitish powdery efflorescence, without distinct odour, and never pigmenting the medium on which it is grown and not fermenting or peptonising milk. Non-pathogenic for monkeys and other laboratory animals.'

5. *Inoculation in Animals*. All attempts to infect monkeys, white rats, gerbils, rabbits and pigeons by various methods of inoculation have, so far, failed in our hands.

6. *Comparisons*. In the historical part of this paper we invited attention to seven species of *Nocardia* and two species of *Cohnistreptothrix* known to cause Actinomycosis, and with these our Sudanese variety must be compared, but, before so doing, we must invite attention to the *Black Actinomycosis* of Babès and

Mironescu, of which the causal fungus has not been cultivated, and, therefore, its generic classification is doubtful.

In the year 1888, Babès, in Roumania, met with a case resembling an Actinomycosis, in that the pus, escaping via a fistula from a deep abscess, showed small black grains with actinomycotic clubs. The pus also contained pyogenic cocci.

In 1910, he and Mironescu met with a second case of a similar nature, also in Roumania. In this case a retro-bulbar abscess opened by means of a fistula in the upper eyelid. Eventually the eye became diseased and had to be removed, an abscess formed in the brain, and the man died. In the pus from these abscesses they found pyococci and black grains, which latter were curious in that they were not due solely to the contained fungus but were formed by the connective tissue being changed into black masses.

The fungus was composed of thick Gram-negative hyphae, measuring two microns in diameter and *falsely* branched at acute or right angles. They appear to have had a distinct membrane but not a thick hyphal wall, and septa are not described or illustrated.

The authors lay stress upon the thickness of the hyphae and the false branching as distinguishing this fungus from a *Nocardia* (*Streptothrix*). All attempts to grow this causal organism or to infect animals were negative, and, therefore, they are unable to classify the fungus, which they doubtfully think may be Cladothrix.

When the illustrations are examined, the fungus is seen to resemble a *Nocardia* with thick hyphae, especially as true branching is depicted in fig. 3, but, be that as it may, the descriptions given by the authors agree with our definitions of 'Mycetoma,' 'Granum' and 'Actinomycosis,' and, therefore, though the systemic position of the fungus is doubtful, still the disease is an Actinomycosis with black grains, and as such must be distinguished from the Sudanese variety.

A method of differentiation of these various Actinomycoses is set forth in the following Table :—

A. Grains black, fungus not cultivated	*Babès and Mironescu's Actinomycosis*
B. Grains white, yellow, reddish yellow, yellowish red or red, fungus cultivated :—	
I. Cultivation difficult, grow best anaërobically, arthrospores absent	Genus 1 *Cohnistreptothrix*
a. Clubs present :—	
1. Grains yellow	1. *israeli*
2. Grains very small and white	2. *thibiergei*
II. Cultivation easy, grow best aërobically, arthrospores present	Genus 2 *Nocardia*
a. Clubs present	3. *bovis*
b. Clubs absent :—	
1. Grains surrounded by a hard sheath, insoluble in liquor potassae and eau de Javelle	4. *somaliensis*
2. Grains without such a sheath :—	
G^{i}. Growth on gelatine absent	5. *krausei*
G^{ii}. Growth on gelatine present :—	
M. Inspissated blood serum liquefied :—	
x. Pathogenic for laboratory animals, growth on potato white, medium becomes greenish in colour	6. *garteni*
y. Non-pathogenic for laboratory animals, growth on potato yellowish or buff colour, medium unchanged :—	
r^{i}. Gelatine liquefied, and growths not convoluted	7. *liquefaciens*
r^{ii}. Gelatine not liquefied, and growths markedly convoluted	8. *convoluta*
N. Inspissated blood serum not liquefied :—	
x. Growths yellowish orange to brick red	9. *asteroides*
y. Growths at first whitish, later pink	10. *indica*

By this Table it will be observed that *N. liquefaciens* and *N. convoluta* come into close relationship, but in addition to the former belonging to the *Majora* and the latter to the *Minora* sub-

sections of the *Parasitic Section*, the following differences may be noted :—

Reaction	*N. liquefaciens*	*N. convoluta*	Comparison
Conditions of growth	Obligatory aërobe	Facultative anaërobe	Different
Gelatine	Liquefaction begins 4th or 5th day	Not liquefied	Different
Colour of growths	Yellow becoming reddish yellow	Light to warm buff	Different
Medium	In old cultures tinted dark yellow	Not tinted	Different
Potato	Small yellow colonies in two days	Convoluted growth in two days	Different
Amount of growth	Considerable and not restricted	Usually restricted	Different

Therefore, we conclude that *N. convoluta* can be easily distinguished from *N. liquefaciens*, and that it gives rise to a separate form of human Actinomycosis, of which the following table contains a list of the varieties of this disease as known to us :—

A. With White, Yellow or Red Grains

1. *Carter's Actinomycosis* caused by *Nocardia indica* (Kanthack 1893)
2. *Acland's Actinomycosis* caused by *Nocardia bovis* (Harz 1877)
3. *Israel's Actinomycosis* caused by *Cohnistreptothrix israeli* (Kruse 1896)
4. *Eppinger's Actinomycosis* caused by *Nocardia asteroides* (Eppinger 1890)
5. *Hesse's Actinomycosis* caused by *Nocardia liquefaciens* (Hesse 1892)
6. *Krause's Actinomycosis* caused by *Nocardia krausei* (Chester 1901)
7. *Bouffard's Actinomycosis* caused by *Nocardia somaliensis* (Brumpt 1906)
8. *Garten's Actinomycosis* caused by *Nocardia garteni* (Brumpt 1908)

9. *Ravaut and Pinoy's Actinomycosis* caused by *Cohnistreptothrix thibiergei* Ravaut and Pinoy 1909
10. *Chalmers and Christopherson's Actinomycosis* caused by *Nocardia convoluta* Chalmers and Christopherson 1916

B. With Black Grains

11. *Babès and Mironescu's Actinomycosis* with unclassified fungus

SUMMARY

We believe that we have found a new form of Actinomycosis in man in the Sudan, and that the causal fungus is new in man and also new to science, and, therefore, we name it *Nocardia convoluta*, which makes the number of different varieties of the Actinomycotic form of Mycetomas known to exist in man eleven in all.

Diagnosis can only be effected by finding and cultivating the grain, associated with a study of its mycology and that of the cultures therefrom, together with a comparison of the results so obtained with the tables given above.

The treatment which cured this condition was the complete removal of the whole growth, i.e. the removal of all the fungal elements.

ACKNOWLEDGMENTS

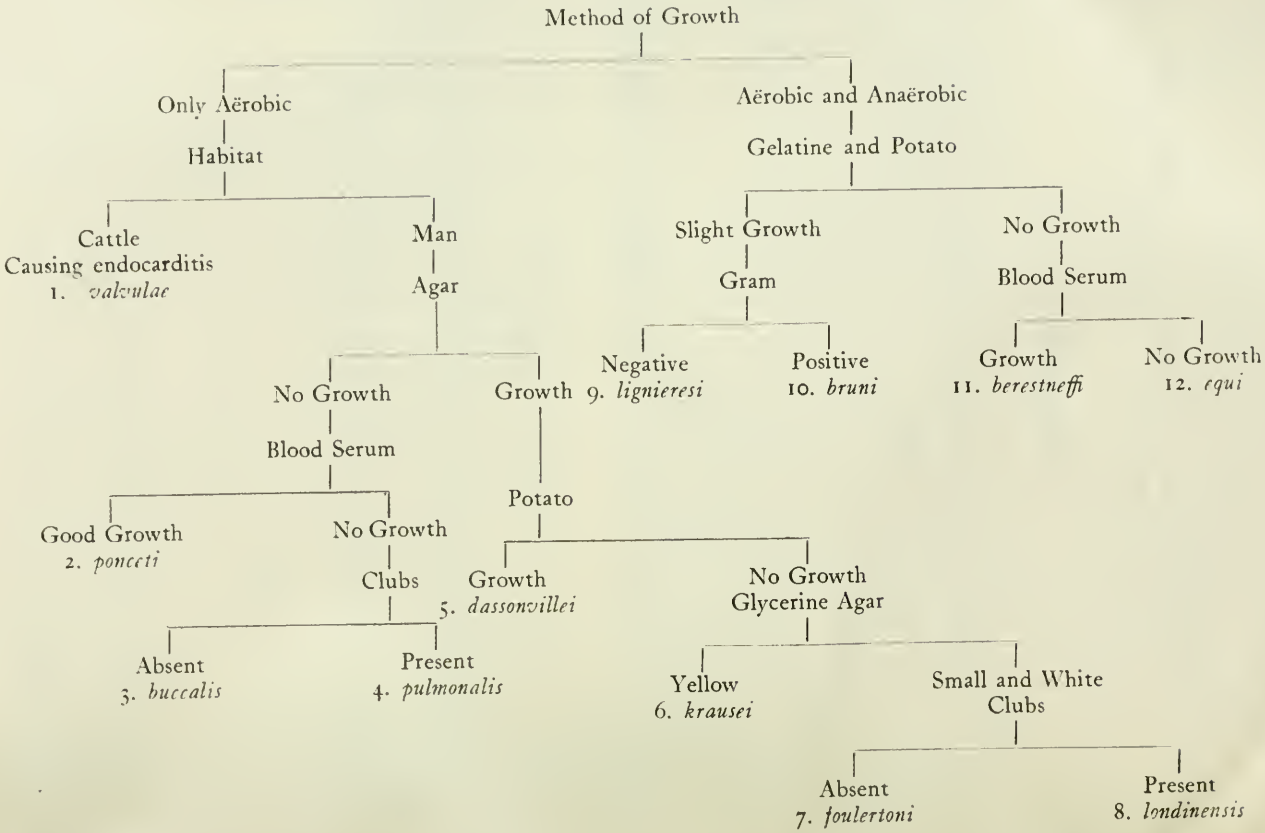
It gives us much pleasure to acknowledge the kindness received from Dr. Asland, and especially from Dr. Foulerton, during the preparation of this paper. The latter most generously supplied us with cultures wherewith to make comparisons with our fungus.

Khartoum,

June 1st, 1916.

TABLE III

Diagnostic Table of the species of the Section *Parasitica*, Sub-section *Brevis*



List II

Synonyms of the species shown in Table III

1. *Nocardia valvulae* (Luginger 1904)
   Streptothrix valvulae destruens bovis Luginger 1904

2. *Nocardia ponceti* Verdun 1912

3. *Nocardia buccalis* (Roger, Bory and Sartory 1909)
   Oospora buccalis Roger, Bory and Sartory 1909
   Nec *Streptothrix buccalis* Goadby 1903

4. *Nocardia pulmonalis* (Roger, Bory and Sartory 1909)
   Oospora pulmonalis Roger, Bory and Sartory 1909

5. *Nocardia dassonvillei* Brocq-Rousseu 1907
   Gasperini's Streptothrix 1890

6. *Nocardia krausei* (Chester 1901)
   Streptothrix krausei Chester 1901

7. *Nocardia foulertoni* new name
   Streptothrix hominis Foulerton 1902
   Streptothrix hominis I Foulerton 1906

8. *Nocardia londinensis* new name
   Streptothrix hominis II Foulerton 1906

9. *Nocardia lignieresi* (Brumpt 1910)
   Actinobacillus lignieresi Brumpt 1910

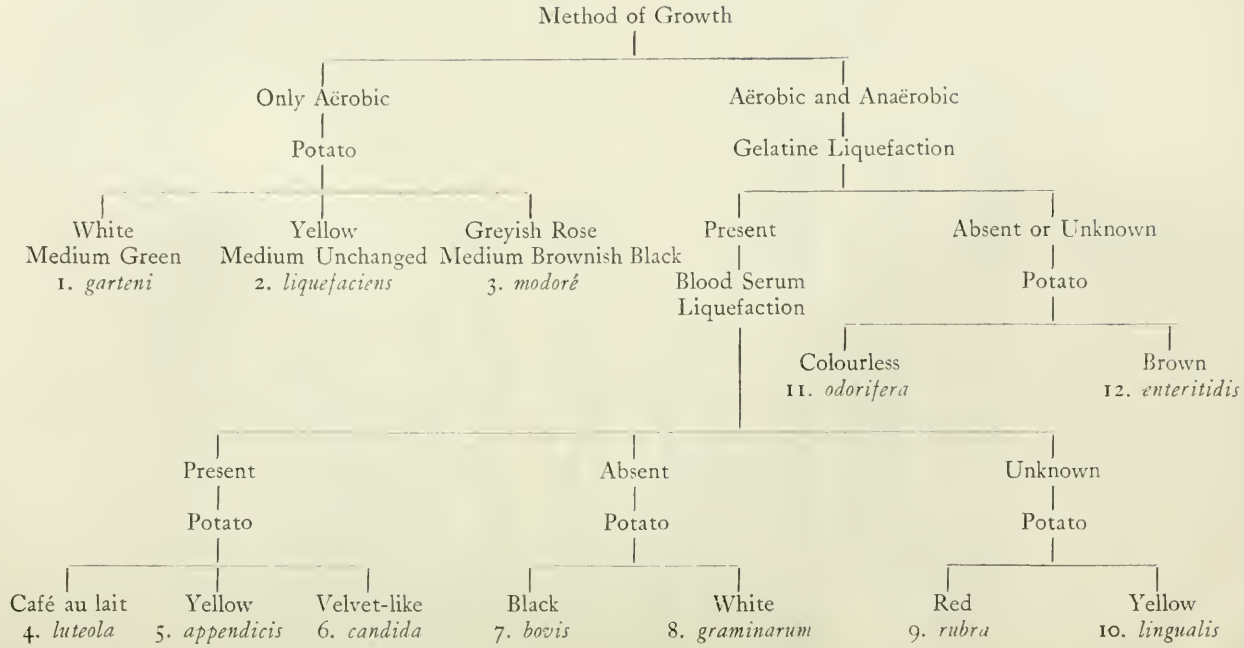
10. *Nocardia bruni* new name
    Streptothrix hominis Hayo Bruns 1899

11. *Nocardia berestneffi* new name
    Streptothrix cases 1 and 2 Berestneff 1897

12. *Nocardia equi* (Dean 1900)
    Streptothrix from a horse of Dean 1900
    Probably the organism described by Norris and Larkin should come here, but we have been unable to see a full description.

TABLE IV

Diagnostic Table of the species of the Section *Parasitica*, Sub-section *Majora*



List III

Synonyms of the species tabulated in Table IV

1. *Nocardia garteni* (Brumpt 1910)
   Cladothrix liquefaciens II Garten 1895
   Discomyces garteni Brumpt 1910
2. *Nocardia liquefaciens* (Hesse 1892)
   Cladothrix liquefaciens Hesse 1892
   Streptothrix liquefaciens (Hesse 1892)
   Streptothrix buccalis Goadby 1903 nec Roger, Bory and Sartory 1909
3. *Nocardia modoré* Thiry 1897
   Cladothrix modoré Thiry 1897
   Cladothrix polychromes Thiry 1897
   Actinomyces rubidaureus Lachner-Sandoval 1898
4. *Nocardia luteola* (Foulerton 1910)
   Streptothrix luteola Foulerton 1910
5. *Nocardia appendicis* new name
   Streptothrix hominis III Foulerton 1910
   Streptothrix hominis IV Foulerton 1906
6. *Nocardia candida* (Petruschky 1898)
   Streptothrix candida Petruschky 1898
   Streptothrix gedanensis II Petruschky 1898
   Streptothrix lathridii Petruschky 1898
7. *Nocardia bovis* (Harz 1877)
   Actinomyces bovis Harz 1877
   Bacterium actino-cladothrix Affanassieff 1888
   Actinomycosis hominis (Affanassieff 1888)
   Nocardia actinomyces de Toni and Trevisan 1889
   Streptothrix actinomyces Rossi-Doria 1891
   Oospora bovis Sauvageau and Radais 1892
   Actinomyces bovis sulphureus Gasperini 1894
   Cladothrix actinomyces Macé 1897
   Discomyces bovis Blanchard 1900
   Streptothrix hominis III Foulerton 1905 nec Foulerton 1910
   Streptothrix hominis IV Foulerton 1910 nec Foulerton 1906
8. *Nocardia graminarium* (Berestneff 1897)
   Streptothrix graminarium Berestneff 1891
9. *Nocardia rubra* Carabó 1894
   Streptothrix rubra Kruse 1896
   Nec *Actinomyces ruber* Krainsky 1914
10. *Nocardia lingualis* (Weibel 1888)
    Vibrio lingualis Weibel 1888
    Spirosoma lingualis Migula 1892
    Streptothrix lingualis Bajardi 1900
11. *Nocardia odorifera* (Rullman 1898)
    Cladothrix odorifera Rullman 1898 in sputum not in air
12. *Nocardia enteritidis* (Pottien 1902)
    Streptothrix enteritidis Pottien 1902

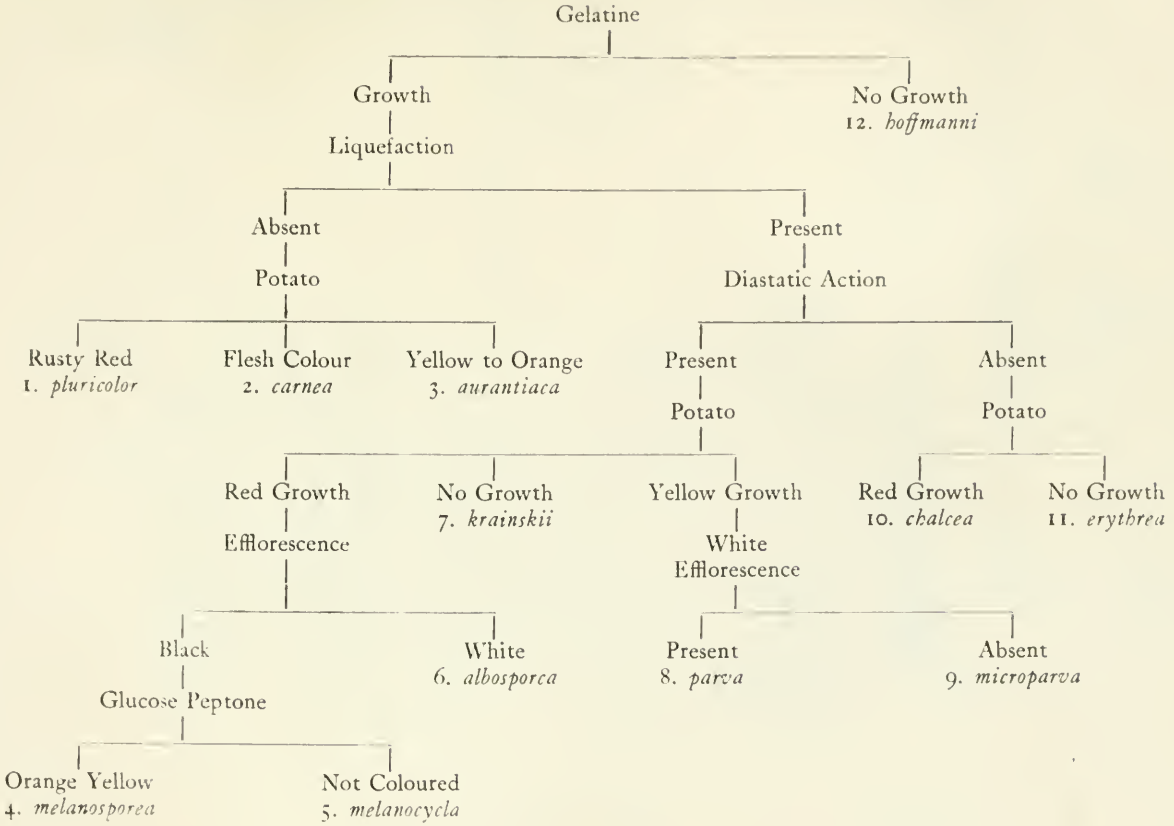
TABLE V

Diagnosis of the Sub-sections of the Section *Nocardia saprophytica*

Sub-section 1—*Majora*	Sub-section 2—*Minora*
1. Grow freely under artificial conditions at 22° C., and generally at 37° C., with a few exceptions.	1. Grow moderately under artificial conditions at 22° C. and 37° C.
2. Growth usually large and spreading.	2. Growth usually moderate and circumscribed.
3. Development of aerial hyphae marked by a bright chalky efflorescence.	3. Development of aerial hyphae marked by a dull dry powdery appearance.
4. Earthy or mouldy smell often present in the cultures.	4. Earthy or mouldy smell either faint or absent.
5. Generally peptonise gelatine and blood serum.	5. Rarely peptonise gelatine and blood serum.
6. Diastatic action often present.	6. Diastatic action usually absent.
7. Hyphal filaments usually coarser, and branching more marked than in next series.	7. Hyphal filaments usually finer, and branching rarer than in the preceding series.

TABLE VI

Diagnostic Table of the species of Section *Saprophytica*, Sub-section *Minora*



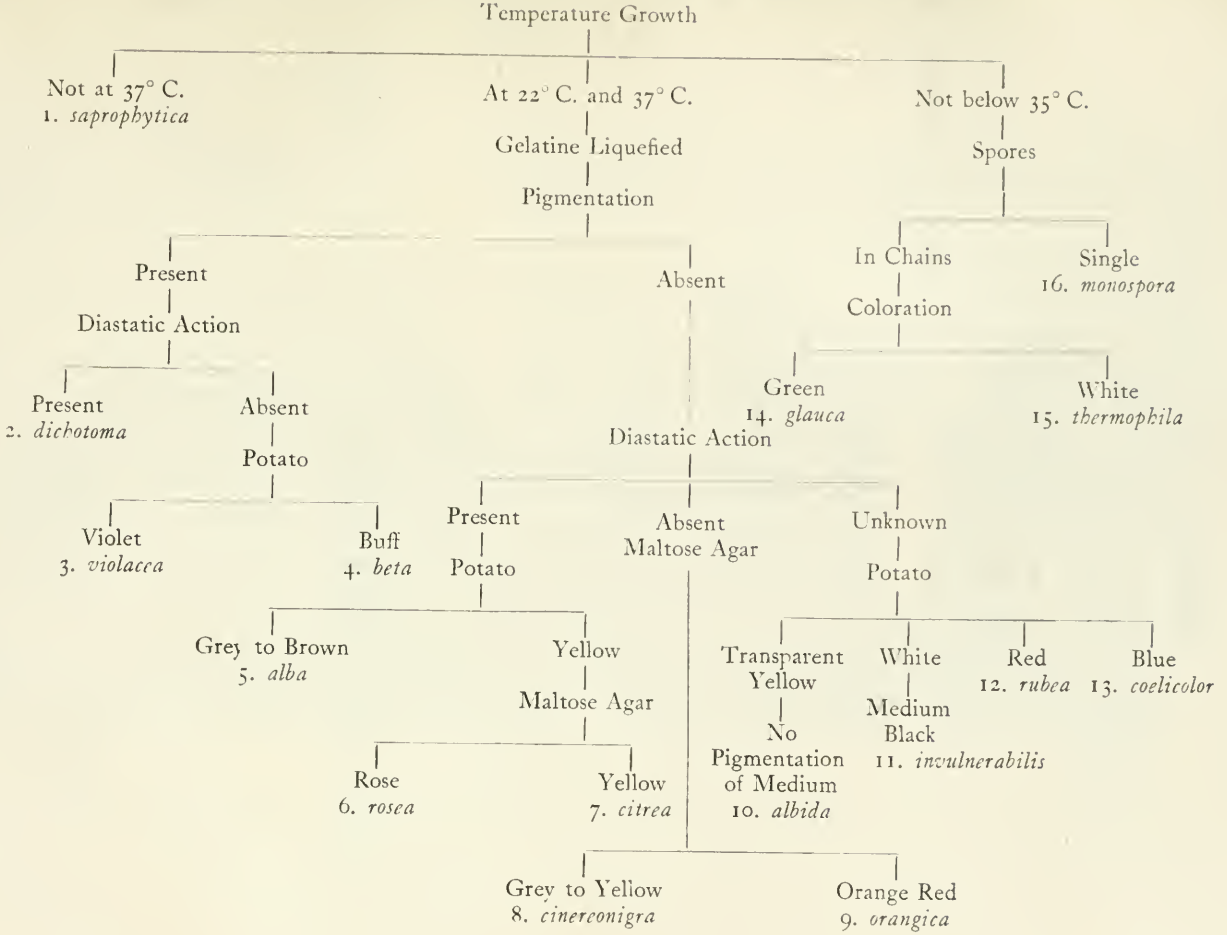
List IV

Synonyms of the species tabulated in Table VI

1. *Nocardia pluricolor* (Terni 1894)
   Streptothrix pluricolor Terni 1894
   Actinomyces gruberi Terni 1894
2. *Nocardia carnea* (Rossi-Doria 1891)
   Streptothrix carneus Rossi-Doria 1891
3. *Nocardia aurantiaca* (Rossi-Doria 1891)
   Streptothrix aurantiacus Rossi-Doria 1891
4. *Nocardia melanosporea* (Krainsky 1914)
   Actinomyces melanosporea Krainsky 1914
5. *Nocardia melanocycla* (Krainsky 1914)
   Actinomyces melanocyclus Krainsky 1914
6. *Nocardia albosporea* (Krainsky 1914)
   Actinomyces albosporea Krainsky 1914
7. *Nocardia krainskii* new name
   Actinomyces rubra Krainsky 1914 nec Carabó 1894 nec Kruse 1896
8. *Nocardia parva* (Krainsky 1914)
   Actinomyces parva Krainsky 1914
9. *Nocardia microparva* (Krainsky 1914)
   Actinomyces microparva Krainsky 1914
10. *Nocardia chalcea* (Foulerton 1905)
    Streptothrix chalcea Foulerton 1905
11. *Nocardia erythrea* (Foulerton 1910)
    Streptothrix erythrea Foulerton 1910
12. *Nocardia hoffmanni* (Gruber 1891)
    Micromyces hoffmanni Gruber 1891

TABLE VII

Diagnostic Table of the species of the Section *Saprophytica*, Sub-section *Majora*



List V

Synonyms of the species tabulated in Table VII

1. *Nocardia saprophytica* (Foulerton 1902)
   Streptothrix leucea saprophytica Foulerton 1902
2. *Nocardia dichotoma* (Macé 1888)
   Cladothrix dichotoma Macé 1888 nec Cohn 1875
   Streptothrix chromogena Gasperini 1890
   Streptothrix nigra Rossi-Doria 1890
   Oospora metschnikovi Sauvageau and Radais 1892
   Cladothrix brauner Hesse 1892
   Cladothrix odorifer Rullman 1895 nec *C. odorifer* Rullman 1898, parasitic in man
   Streptothrix melanotica Price-Jones 1900
   Streptothrix humifica Beijernick 1900
   Streptothrix nigrescens Foulerton 1902
   Actinomyces erythrochromogenes Krainsky 1914
   Actinomyces diastaticochromogenes Krainsky 1914
   Actinomyces viridochromogenes Krainsky 1914
   Actinomyces flavochromogenes Krainsky 1914
3. *Nocardia violacea* (Rossi-Doria)
   Streptothrix violacea Rossi-Doria 1891
   Actinomyces violaceus Berestneff 1897
   ? *Actinomyces alni* Peklo 1910
   ? *Actinomyces myricae* Peklo 1910
4. *Nocardia beta* (Price-Jones 1900)
   Streptothrix beta Price-Jones 1900
5. *Nocardia alba* (Rossi-Doria 1891)
   Streptothrix alba Rossi-Doria 1891
   Actinomyces chromogenes B Gasperini 1890
   Streptothrix I, II and III Almquist
   Actinomyces albus Lehmann and Neumann
   Oospora guiguardi Sauvageau and Radais 1892
   Actinomyces albus Gasperini 1890
   Oospora doriae Sauvageau and Radais 1892
   Streptothrix foersteri Gasperini 1890 nec Cohn
   Streptothrix leucea Foulerton 1902
   Streptothrix alpha Price-Jones 1900
   Streptothrix pyogenes Caminiti 1907
   Actinomyces grisea Krainsky 1914
   Actinomyces diastatica Krainsky 1914
   Actinomyces cellulosae Krainsky 1914
   Actinomyces nivea Krainsky 1914
6. *Nocardia rosea* (Krainsky 1914)
   Actinomyces roseus Krainsky 1914
7. *Nocardia citrea* (Krainsky 1914)
   Actinomyces griseoflavus Krainsky 1914
   Actinomyces flavus Krainsky 1914
   Streptothrix flava Sanfelice 1904
   Streptothrix flava Brins 1899

8. *Nocardia cinereonigra* (Berestneff 1897)
   Streptothrix cinereonigra aromatica Berestneff 1897
9. *Nocardia orangica* (Berestneff 1897)
   Streptothrix orangica Berestneff 1897
10. *Nocardia albida* (Rossi-Doria 1891)
   Streptothrix albido-flava Rossi-Doria 1891
   Actinomyces farcinicus Rossi-Doria 1891
   Nocardia farcinica Rossi-Doria 1891
11. *Nocardia invulnerabilis* (Acosta and Grande Rossi 1893)
   Cladothrix invulnerabilis Acosta and Grande Rossi 1893
12. *Nocardia rubea* new name
   Actinomyces ruber (no name)
   Nec *Actinomyces ruber* Krainsky 1914
   Nec *Streptothrix rubra* Casabó 1894
   Nec *Streptothrix rubra* Kruse 1896
13. *Nocardia coelicolor* (R. Müller 1904)
   Streptothrix coelicolor R. Müller 1904
   Streptothrix coelicolor Schurman 1909
14. *Nocardia glauca* (Lehmann and Schulze)
   Actinomyces glaucus Lehmann and Schulze
15. *Nocardia thermophila* (Gilbert 1904)
   Actinomyces thermophilus Gilbert 1904
   Cladothrix thermophilis Kedzior
   Actinomyces thermophilus Berestneff 1891
16. *Nocardia monospora* (Schulze 1908)
   Actinomyces monosporus Schulze 1908

List VI

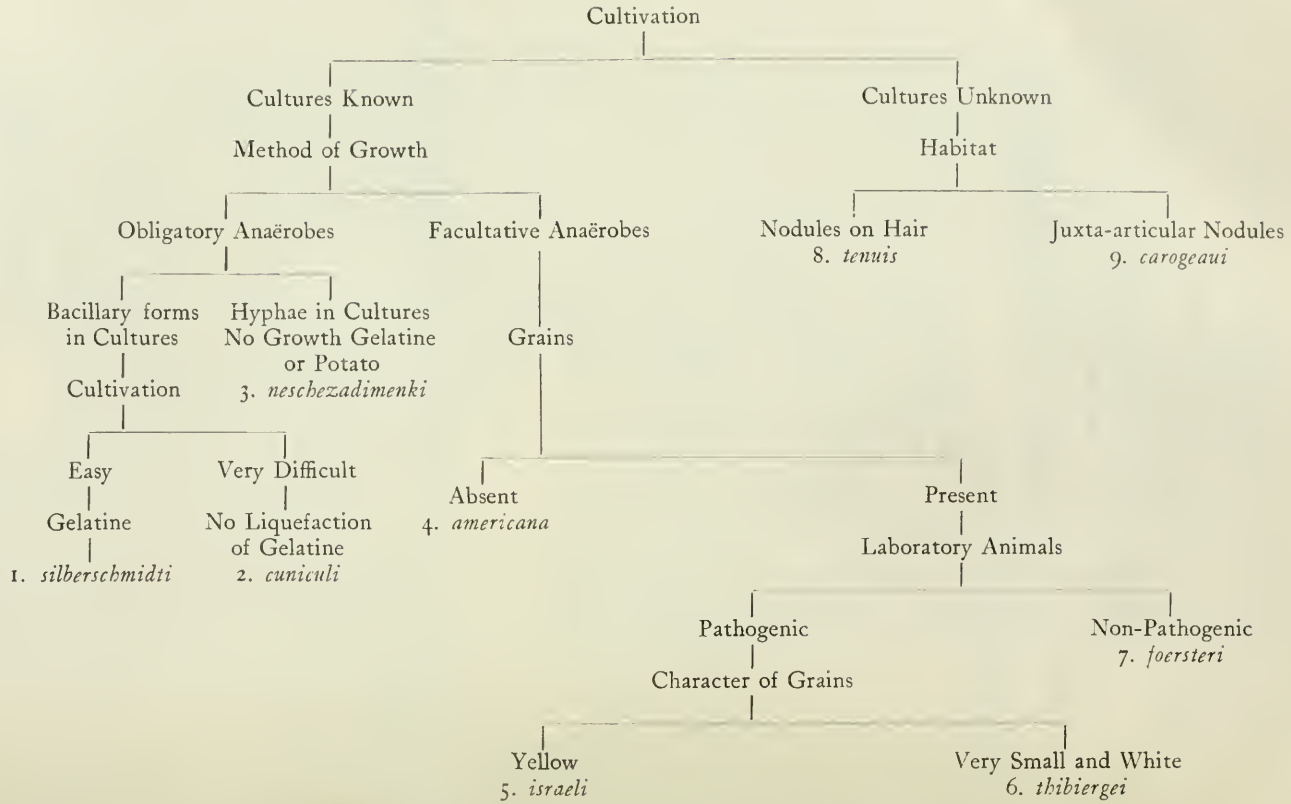
Incertae Sedis

In this list we have included the forms concerning which we have been unable to obtain full information and have, therefore, been unable to classify according to the above tables :—

1. *Actinomyces lacertae* Terni 1891
2. *Streptothrix pseudotuberculosa* Flexner 1898
3. *Streptothrix* of Bonvicini 1899
4. *Streptothrix polychromogenes* Vallée 1900
5. *Actinomyces bicolor* Trollender 1903
6. *Nocardia liguire* Urizer 1904
7. *Actinomyces verrucosus* Adler 1904
8. *Nocardia lasserei* Verdun 1912
9. *Nocardia decussata* Langeron and Chevalier 1912
10. *Actinomyces musculorum suis* Duncker
11. *Actinomyces pseudotuberculosis* Hamm and Keller
12. *Discomyces holmesi* mentioned by Goedelst
13. *Streptothrix gelatinosa* } mentioned by Peklo
14. *Streptothrix aquatilis* } mentioned by Peklo
15. *Streptothrix lehmann* } mentioned by Peklo
16. *Streptothrix chondri* Olsen 1897 } mentioned by Peklo

TABLE VIII

Diagnostic Table of the species *which may possibly* belong to the genus *Cohnistreptothrix* Pinoy 1911



List VII

Synonyms of the species tabulated in Table VIII

1. *Cohnistreptothrix silberschmidti* new name
   This name is given to distinguish the obligatory anaërobic Streptothrix found by Silberschmidt in 1900, in Dacryocystitis, and described in the *Centralblatt für Bakteriologie*, XXVII, and further cases in *Zeitschrift für Hygiene* (1901), XXXVII.

2. *Cohnistreptothrix cuniculi* (Schmorl 1891)
   Streptothrix cuniculi Schmorl 1891
   Actinomyces cuniculi Gasperini 1894
   Streptothrix necrophora Kitt 1906
   ? *Bacillus necroscos* Salmonsen
   ? *Necrosis bacillus* of Bang
   ? *Bacillus diphtheriae vitulorum* Flügge
   ? *Bacillus necrophorus* Flügge

3. *Cohnistreptothrix neschezadimenki* new name
   This name is given to distinguish the obligatory anaërobic Streptothrix found by Neschezadimenko, in 1908, in human pus, and described in the *Centralblatt für Bakteriologie*, XLVI.
   ? *Coccobacillus pseudo-actinomycosis polymorphus* Berestneff 1898

4. *Cohnistreptothrix americana* new name
   This name is given to distinguish the Streptothrix which only grows under partial anaërobic and aërobic conditions, and obtained from a liver abscess by Bloomfield and Bayne-Jones in 1915, and described in *Johns Hopkins Hospital Bulletin*, XXVI, No. 292.

5. *Cohnistreptothrix israeli* (Kruse 1896)
   Streptothrix israeli Kruse 1896
   Streptothrix spitzi Lignières 1903
   Possibly the Streptothrices described by Doyen in 1891, by Jurinka in 1896, and some of those by Silberschmidt in 1901, by Schukewitsch in 1902, by Doepke in 1903, and by Wright in 1904.

6. *Cohnistreptothrix thibiergei* (Ravaut and Pinoy 1909)
   Discomyces thibiergei Ravaut and Pinoy 1909

7. *Cohnistreptothrix foersteri* (Cohn 1874)
   Streptothrix foersteri Cohn 1874
   Leptothrix oculorum Sorokin 1881
   Oospora foersteri Sauvageau and Radais 1892
   Streptothrix aureus Du Bois de Saint Sévérin 1895
   Streptothrix foersteri Kruse 1896
   The aërobic Streptothrix of Silberschmidt obtained from a case of Dacryocystitis 1901.
   ? *Streptothrix radiatus* Namyslowski 1909
   ? *Streptothrix cerebriformis* Namyslowski 1909

8. *Cohnistreptothrix tenuis* (Castellani 1911)
   Nocardia tenuis Castellani 1911

9. *Cohnistreptothrix carogeaui* (Cougerot 1909)
   Discomyces carougeaui Cougerot 1909
   Nocardia carougeaui Castellani and Chalmers 1913

REFERENCES

Arranged in alphabetical sequence

The most valuable bibliographies are those contained in Acland (1906), and Musgrave, Clegg and Polk (1908)

Acland (1886). *Transactions of the Pathological Society of London.* London.

——— (1906). Allbutt and Rolleston's System of Medicine, Vol. II, Part I, pp. 324-343. London.

Archibald (1911). *Fourth Report of the Wellcome Tropical Research Laboratories*, A, Medical, pp. 337-342. London.

Babès (1913). Kolle and Wassermann's *Handbuch der Pathogenen Mikro-organismen*, Vol. V, pp. 363-390. Jena.

Babès and Mironescu (1910). *Centralblatt für Bakteriologie*, I, O, Vol. LV, pp. 108-116. Jena.

Balfour (1911). *Fourth Report of the Wellcome Tropical Research Laboratories*, A, Medical, pp. 365-367. London.

Birt and Leishman (1902). *Journal of Hygiene*, Vol. II, pp. 120-128. Cambridge.

Bloomfield and Bayne-Jones (1915). *Bulletin of the Johns Hopkins Hospital*, p. 230. Baltimore.

Boyce and Surveyor (1894). *Philosophical Transactions of the Royal Society of London*, Vol. CLXXXV, B. London.

Brumpt (1905). *Archives de Parasitologie*, Vol. X, pp. 489-572. Paris.

——— (1913). *Précis de Parasitologie*, pp. 939-944. Paris.

Butterfield (1905). *Journal of Infectious Diseases*, Vol. II, pp. 421-430. Chicago.

Carroll (1905). *Journal of the Royal Army Medical Corps*, Vol. IV, pp. 655-656. London.

Carter, Vandyke (1874). Mycetoma, or the Fungus Disease of India. London.

Castellani and Chalmers (1913). *Manual of Tropical Medicine*, 2nd Edition, pp. 1527-1536. London.

Chalmers and Archibald (1915). Fungi Imperfecti in Tropical Medicine. London.

Chester (1901). *Manual of Determinative Bacteriology*, pp. 360-369. New York.

Clegg and Hobdy (1916). *American Journal of Tropical Diseases*, Vol. III, pp. 534-544. New Orleans.

Coquerel (1866). *Pérical, Pied de Madura, Comptes Rendus de la Société de Biologie*, Series 4th, Vol. II, pp. 191-196. Paris.

Corre (1883). *Archives de Médecine Navale*, pp. 81-137, and 204-224. Paris.

Cranwell, Bachmann, and Del Pont (1909). *Libro de Oro ofrecido Al Prof. Dr. Roberto Wernicke*, pp. 209-232. Buenos-Aires.

Foulerton (1905). *Lancet*, Vol. I, 1, p. 200. London.

——— (1906). *Ibid.*, Vol. I, p. 970. London.

——— (1906). Albutt and Rolleston's System of Medicine, Vol. II, 1, pp. 302-324. London.

——— (1910). Streptotrichoses and Tuberculosis (Milroy Lectures). London.

——— (1912). *British Medical Journal*, February 10, p. 300. London.

Foulerton and Jones (1902). *Transactions of the Pathological Society of London*, Vol. LIII, pp. 56-127. London.

Fülleborn (1911). *Archiv für Schiffs und Tropenhygiene*, Vol. XV, pp. 131-132. Leipzig.

Garten (1895). *Deutsche Zeitschrift für Chirurgie*, pp. 257-284.

Gilbert (1904). *Zeitschrift für Hygiene und Infectionen Krankheiten*, Vol. XLVII, pp. 383-405. Leipzig.

Goadby (1903). Mycology of the Mouth, pp. 200-205. London.

Griffith (1916). *Journal of Hygiene*, Vol. XV, 2, p. 195. Cambridge.

Haass (1906). *Centralblatt für Bakteriologie*, O, Vol. I, pp. 180-186. Jena.

Harz (1877-78). *Jahresberichte der Koeniglichen Central Thierarzneischule*, München.

Hesse (1892). *Deutsche Zeitschrift für Chirurgie*, pp. 274-307.

Henry (1910). *Journal of Pathology and Bacteriology*, Vol. XIV, pp. 164-172. Cambridge.

Kanthack (1893). *Journal of Pathology and Bacteriology*, Vol. I, pp. 140-159. Edinburgh.

——— (1896). *St. Bartholomew's Hospital Reports.*

Koch and Stutzer (1911). *Zeitschrift für Hygiene*, Vol. LXIX, pp. 17-24. Leipzig.

Krainsky (1914). *Centralblatt für Bakteriologie*, II, Vol. XLI, pp. 649-688. Jena.

Kruse (1896). *Flügge's Die Mikro-organismen*, 2nd Edition, Vol. II, pp. 48-66. Leipzig.

Lehert (1857). *Atlas d'Anatomie pathologique.*

Lignières and Spitz (1904). *Centralblatt für Bakteriologie*, I, O, Vol. XXXV, pp. 294-308, 452-458. Jena.

Macé (1913). *Traité de Bacteriologie*, Vol. II, pp. 720-760. Paris.

Mertens (1903). *Zeitschrift für Hygiene*, Vol. XLII, pp. 45-89. Leipzig.

Meyen (1827). *Linnaea*, Vol. II, p. 433.

Münter (1913). *Centralblatt für Bakteriologie*, II, Vol. XXXVI, pp. 365-381. Jena.

Musgrave and Clegg (1907). *Philippine Journal of Sciences*, Medical, Vol. II, p. 477. Manila.

——— ——— (1908). *Ibid.*, Vol. III, p. 447. Manila.

Peklo (1910). *Centralblatt für Bakteriologie*, Abteilung II, Vol. XXVII, pp. 451-579. Jena.

Petrusky (1913). Kolle and Wassermann's *Handbuch der Pathogenen Mikro-organismen*, Vol. V, pp. 267-300. Jena.

Pinoy (1913). *Bulletin de l'Institut Pasteur*, pp. 929-938, 977-984. Paris.

Plehn (1914). Mense's *Handbuch der Tropenkrankheiten*, 2nd Edition, Vol. II, pp. 234-243. Leipzig.

Poncet and Berard (1898). L'Actinomycose Humaine. Paris.

Rullman (1895). Cladothrix odorifera. München.

Sanfelice (1904). *Centralblatt für Bakteriologie*, I, O, Vol. XXXVI, pp. 355-367. Jena.

Sauvageau and Radais (1892). *Annales de l'Institut Pasteur*, pp. 242-273. Paris.

Schlegel (1913). Kolle and Wassermann's *Handbuch der Pathogenen Mikro-organismen*, Vol. V, pp. 301-365. Jena.

Wright (1904). *Journal of Medical Research*, Vol. XIII, pp. 349-404. Boston.

EXPLANATION OF PLATES

Most of these illustrations may, with advantage, be examined by means of a reading lens.

Plate VIII

Fig. 1. Sudanese Actinomycotic Mycetoma. × 1·5 diameters. Photograph.

Fig. 2. Eosinophile bodies, often called Fuchsin—or Botryomycotic bodies. × 1,440 diameters. Photomicrograph.

Fig. 3. A grain removed from the Mycetoma depicted in fig. 1. × 20 diameters. Photograph.

Fig. 4. Aërobic growth for five days at 30° C. on Sabouraud's preservative medium, of the fungus *Nocardia convoluta*, derived from grains taken from the Mycetoma depicted in fig. 1. Photograph.

Fig. 5. *N. convoluta.* Growth on clear maltose agar, watch-glass method, for eight days at 37° C. Photograph.

Fig. 6. Fresh preparation in normal saline from the grain depicted in fig. 3. Showing nocardial hyphae. Photomicrograph.

Fig. 7. *N. convoluta.* Growth on potato for four days at 37° C. Photograph.

Fig. 8. *N. convoluta.* Aërobic growth on Sabouraud's preservative medium for eight days at 30° C. Photograph.

Fig. 9. *N. convoluta.* Anaërobic growth on Sabouraud's preservative medium for twelve days at 30° C. Photograph.

Fig. 10. *N. convoluta.* Growth on inspissated ox-blood serum for seven days at 30° C. Photograph.

Fig. 11. *N. convoluta.* Growth on inspissated ox-blood serum for twelve days at 30° C., showing commencing liquefaction of the medium. Photograph.

Fig. 1

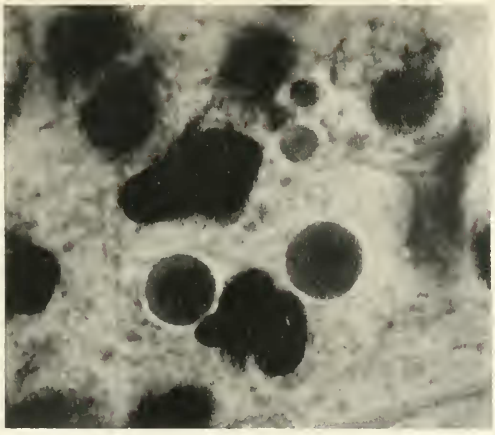


Fig. 2

Fig. 3

Fig. 4

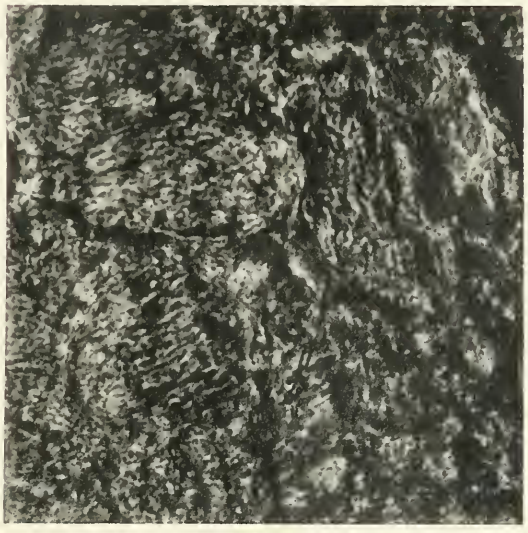


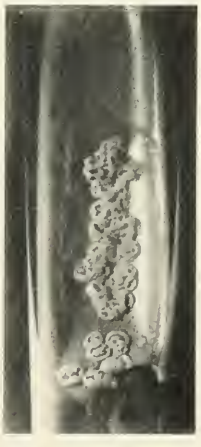
Fig. 6

Fig. 7

Fig. 5

Fig. 8

Fig. 9



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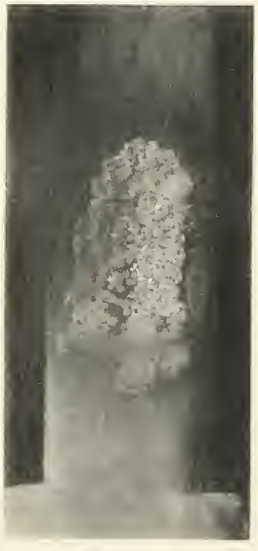


Fig. 11

Plate IX

Fig. 12. General view of an old fungal mass. × 30 diameters. Photomicrograph.

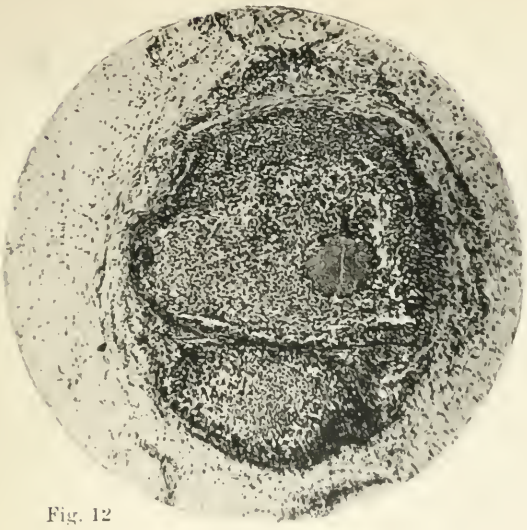
Fig. 13. Vessels, cell masses and fat cells in matrix. × 90 diameters. Photomicrograph.

Fig. 14. Vessel showing peri- and endarteritis. × 400 diameters. Photomicrograph.

Fig. 15. Cells accumulated near a vessel, as depicted in fig. 13. × 500 diameters. Photomicrograph.

Fig. 16. Fungus escaping from giant cell. × 400 diameters. Photomicrograph.

Fig. 17. Young fungal mass. Note the remains of the giant cell and also the polymorphonuclear cells of the fibro-cellular sheath. × 400 diameters. Photomicrograph.



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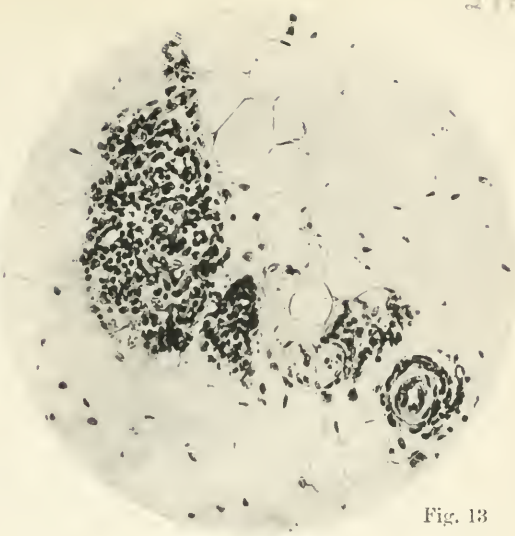

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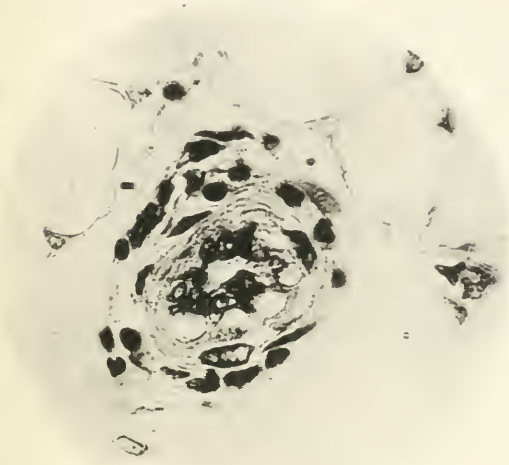

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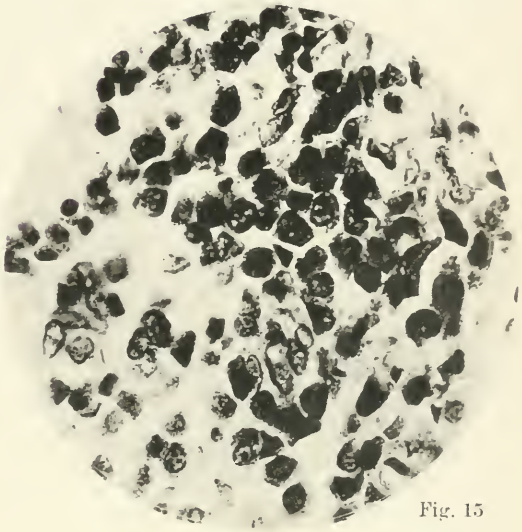

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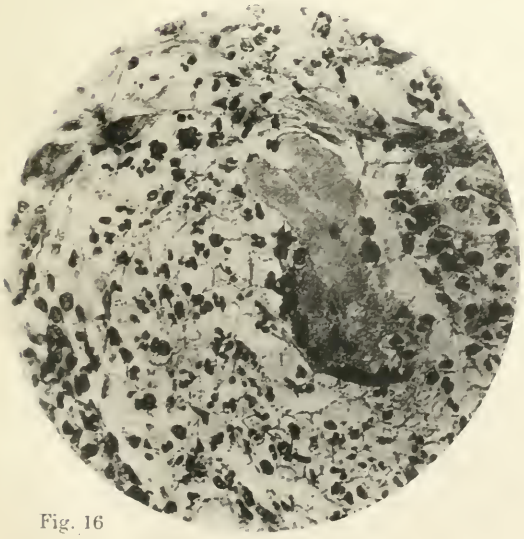

Fig. 16

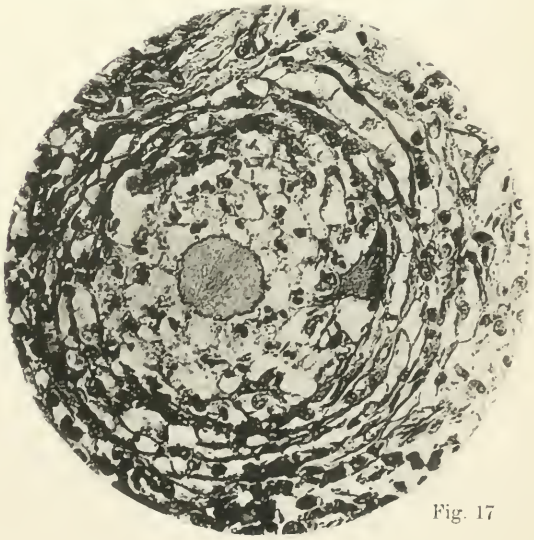

Fig. 17

Plate X

Fig. 18. Higher power view of fig. 12. × 82 diameters. Photomicrograph.

Fig. 19. Giant cells containing portion of the fungus. × 940 diameters. Photomicrograph.

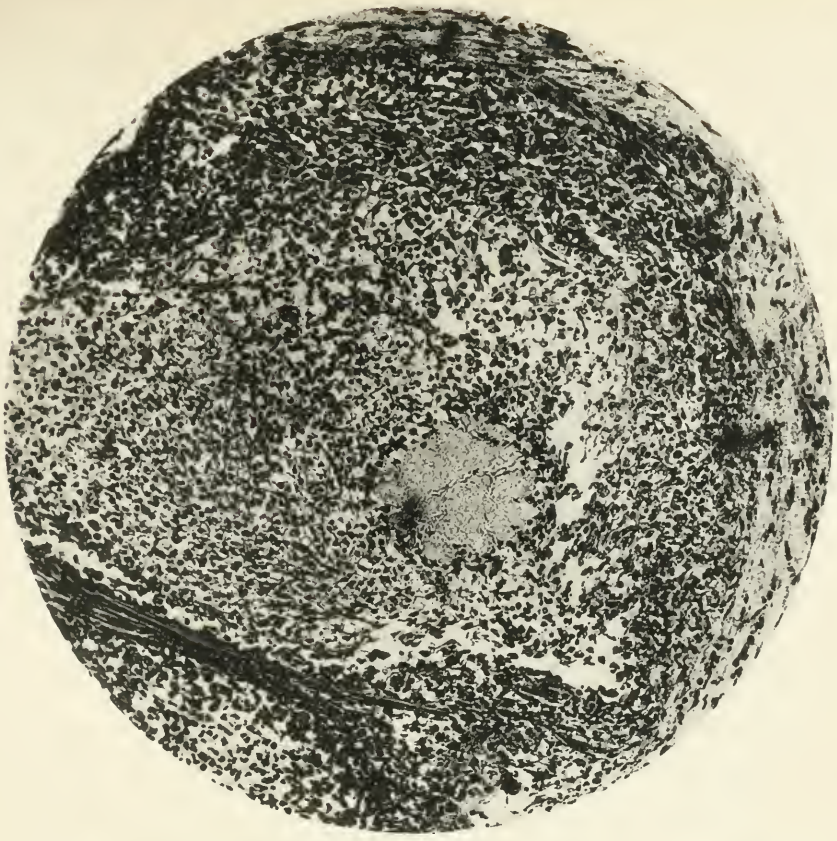

Fig. 18

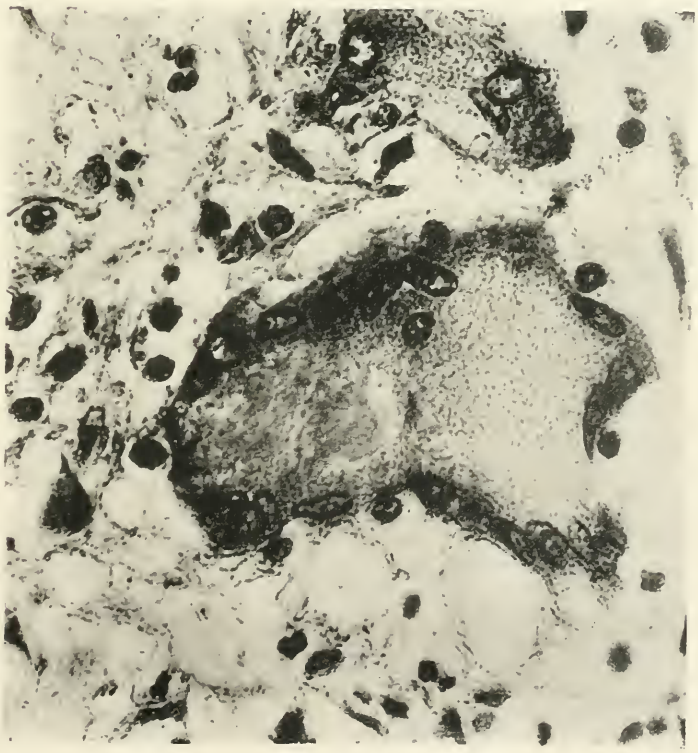

Fig. 19

A SUDANESE ACTINOMYCOSIS

Plate XI

Fig. 20. *N. convoluta.* Hypha showing beading, also commencing separation into three portions. Blood serum culture at 30° C. × 1,500 diameters. Photomicrograph.

Fig. 21. *N. convoluta.* Hypha showing branching. Note also forms with commencing arthrospores, also bacillary and coccal forms. Blood serum culture at 30° C. × 1,500 diameters. Photomicrograph.

Fig. 22. *N. convoluta.* Young hypha showing cytoplasm and beading. Blood serum culture at 30° C. × 2,800 diameters. Photomicrograph.

Fig. 23. *N. convoluta.* Growth on Sabouraud's maltose agar for five days at 37° C. × 1·5 diameters. Photograph.

Fig. 24. *N. convoluta.* Hypha with Gram-positive and Gram-negative lengths; the former filled with cytoplasm, which is lacking in the latter. Blood serum culture. × 1,000 diameters. Photomicrograph.

Fig. 25. *N. convoluta.* Showing breaking up and absorption of the Gram-positive material of the hyphae prior to the stage depicted in fig. 24, which leads to the fragmentation of the hyphae. × 1,000 diameters. Photomicrograph.

Fig. 26. *N. convoluta.* Hyphae with chains of spores.

Fig. 27. *N. convoluta.* Arthrospores.

Fig. 28. *N. convoluta.* Young growth on agar-agar at 30° C. for forty-eight hours. × 4 diameters. Photograph.

Fig. 29. *N. convoluta.* Growth on glucose agar at 30° C. for three days. × 2 diameters. Photograph.

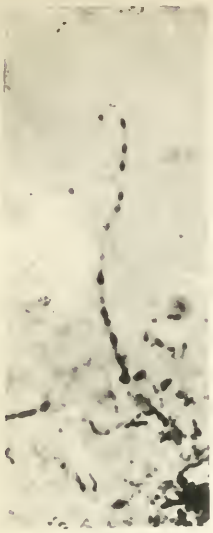

Fig. 20

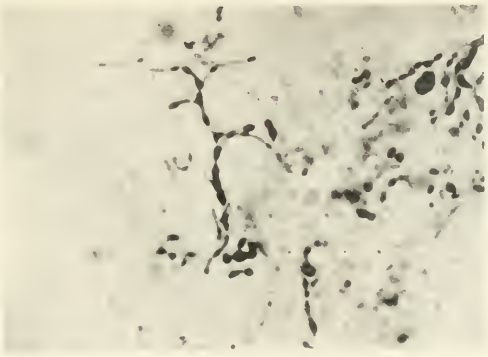

Fig. 21

Fig. 22

Fig. 23

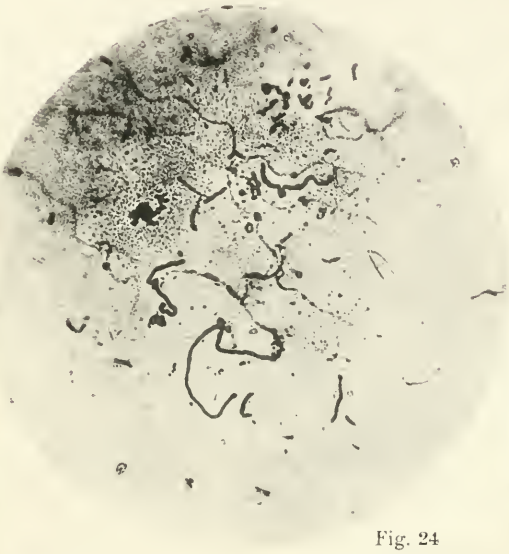

Fig. 24

Fig. 25

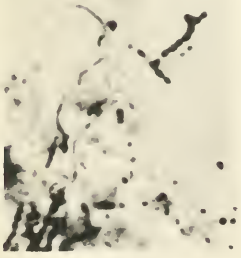

Fig. 26

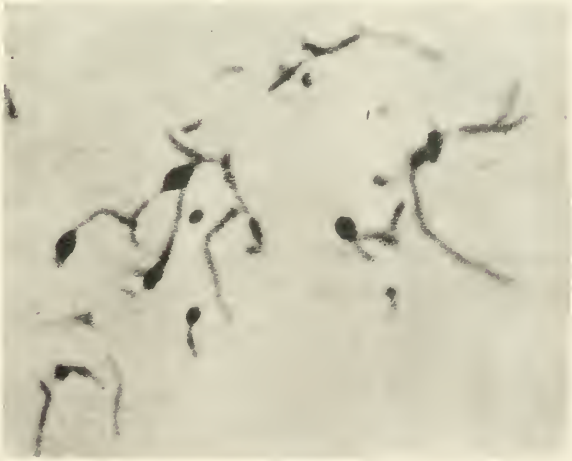

Fig. 27

Fig. 28

Fig. 29

A SUDANESE ACTINOMYCOSIS